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New breast cell lines and cell strains were developed from breast cancer tissues, uninvolved tissues from cancer patients, and normal tissues from reductive mammoplasty patients. Primary cultures were initiated using cell suspensions isolated from tissues and using cellular outgrowth from explanted tissues. Breast cultures were transfected with pRSV-T plasmid or transfected with a plasmid containing mutated *ras* oncogene or were treated with a mutagenic chemical. Various cell isolation methods and new serum-free media were used for developing cell cultures. Twenty cell strains and 10 cell lines (8 epithelial and 2 fibroblastic) were established and added to the breast cell repository. The majority of the epithelial lines have been in continuous culture for at least 20 weeks. The karyotype, cumulative population doubling levels, anchorage-independent growth, and tumorigenicity in nude mice for most of the established cell lines have been documented. Most cell lines possess the H-*ras*, K-*ras*, and p53 genes and express the cytokeratin 8 and 18, BRCA-1, erbB2, and EGFR genes. Two anticancer agents, taxol and 5-fluorouracil, produced significant cytotoxic effects on one of the new cancer cell lines. Genomic DNA was isolated from the epithelial lines and stored in the DNA bank.

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PI - Signature 10/23/97
Date

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INTRODUCTION

This grant (DAMD-17-94-J-4395) was awarded to BRFF in August 1994 for a two year period. BRFF requested a one-year extension without additional funding on July 10, 1996 and the extension was approved on August 1, 1996. As mandated in the extension, a second Annual Report was submitted on September 27, 1996. The final requirement of the extension was the submission of this *Final Report* at the end of the third year.

Nature of the Problem

The main objectives for this grant were (i) to develop a number of new human breast cell lines (the aim was to establish 25 cell lines) from cancer patients of divergent ethnicity and different stages of the disease, and (ii) to operate a full service repository for such cells and genomic DNA in order to make them available to the research community for further breast cancer research. The nature of the work to achieve the two aspects supported by this grant was quite different. Establishment of cell lines required considerable research and development (R&D) work while the implementation of the cell repository required appropriate managerial and administrative effort.

Background of Previous Work

The availability of well-characterized human breast cell cultures capable of long-term growth will stimulate basic and applied breast cancer research. Although it is possible to develop human breast cell cultures which can be grown for a few subcultures, it has been fairly difficult to establish permanent human breast cell lines as evidenced by the small number of such cell lines in existence to date. It is important to identify new therapeutic drugs for breast cancer using cell lines derived from numerous breast tissues. New well-characterized breast cancer cell lines may turn out to be ideal "targets" for developing specific chemotherapeutic agents designed to inhibit breast cell proliferation. To identify the molecular defects which accumulate during the initiation and progression of cancer, it is also essential to study the basic biology, physiology, and biochemistry of normal breast cells. During this grant period, we developed breast cancer cell lines from metastatic breast cancers invaded into the lymph nodes and from primary tumors. In addition, we established normal breast cell lines from normal breast tissues obtained from reductive mammoplasty.

Several breast cancer cell lines have been established during the last 25 years (*inter alia* 2,9,18,42,45,54,64). The most widely used cancer cell lines are MCF-7 (64), T-47D (42), and the various MDA-MB cell lines (9). In addition, several new breast cancer cell lines have been established during the last decade (2,18,45,54). All except one cancer cell line (2) were developed from pleural effusions. The reported cell lines represent just a subset of the cell lines which will be required as models to study different aspects of breast cancer.

Plausible explanations for the difficulty in establishing permanent human breast cell lines include the culture conditions used (75) and the intrinsic inability of human cells to divide indefinitely (30,31). The proliferation and differentiation of human breast epithelial cells are regulated by multiple interacting factors including hormones and growth factors. It is likely that

existing culture media may not provide the optimal environment for the growth of breast cells. Moreover, there are considerable differences between cell types in the tolerance for cell dissociation procedures. Advances in culture media (19,27,34,37,46), tissue fractionation methods (13,65), and cell culture procedures including experimental immortalization of the cells before the onset of senescence (39,40,56,66,72-74) have facilitated development of human mammary epithelial cell lines. Our goal was to isolate new cell lines from breast tissue by applying culture techniques which used gentle cell dissociation methods, minimized the use of serum, used coating mixtures to support the attachment of primary cells to plastic dishes, and used experimental immortalization.

Purpose of the Present Work

The primary objective of this project was to establish, maintain and operate a repository for cell cultures and genomic DNA derived from human breast tissues. The cell collection was to include both cell strains and cell lines established at BRFF using different culture media and experimental manipulations. Towards this goal, breast tissue obtained in the last three years was used to develop cell strains/cell lines. In addition, some of the cell lines developed at BRFF were partially characterized.

Methods of Approach

Three approaches were used to develop cell cultures at BRFF. The first approach utilized classical methods that favor the growth of fibroblastic and/or normal diploid epithelial cells, as well as innovative methods that selectively grow epithelial cells. These epithelial cells were grown in special culture media supplemented with low levels of serum, as well as in serum-free media. Part of the original tissue and the cell strains were cryopreserved at the earliest possible time; cell lines from the same cell strain were expanded and again cryopreserved in larger batches. The second approach to obtain breast cell lines with malignant properties (or transformed phenotype) was to transfect breast cells derived from normal or cancer tissue with a plasmid containing mutated *ras* oncogene. The third approach was to treat normal (non-malignant) breast cells in culture with chemical carcinogens *in vitro.* Although the latter two methods are unorthodox ways of producing breast cancer cells, the resultant cell lines may be useful in understanding the carcinogenic process.

Some of the continuous (immortal) cell lines were characterized with respect to morphology, karyology, gene detection, gene expression, growth rate, immunological detection of protein expression, anchorage-independent growth, and tumorigenic capacity in nude mice.

All of the cell strains/cell lines are stored in the cell repository at BRFF. In addition, DNA isolated from selected cell lines is stored in the DNA bank at BRFF. To insure error-free operation of the cell repository and DNA bank, a body of comprehensive Standard Operating Procedures (SOP) governed the technical aspects of the various tasks and also the logistical aspects of tracking samples obtained from or delivered to investigators.

BODY

Experimental Methods Used

Cell Culture Methods. Two methods were used to set up primary cultures of the breast cancer tissue: (I) plating single-cell suspensions or (ii) attaching small pieces of the tissue as explants on specially treated growth surfaces.

For the first method, single cell suspensions were produced by progressive enzymatic dissociation of the tissue (17) or mechanical "spilling" of the cells (43). The tissue was washed free of the transport medium, minced and suspended in 10 volumes of CTC dissociation medium (contains 0.1% Collagenase (Type I Worthington Biochemicals), 0.05%Trypsin, (Type XI Sigma), and 1% Chicken serum in HEPES Buffered Saline (HBS)) in a conical flask. The minced tissue was incubated at 37°C for 30 minutes, and then isolated cells in suspension were sieved through a cell strainer (Falcon #2350) and collected by centrifugation (100 x *g* for 5 min). Dissociation of the undigested tissue was repeated two or more times and the pooled cell suspension counted and plated as the primary culture. In some cases, the cells from the tissue could be disaggregated by cutting the tissue into small fragments.

For the second method which utilized explant cultures (34,35), the tissue was washed free of the transport medium and cut into 1-2 mm^3 fragments with crossed scalpel blades. These fragments were then transferred to culture dishes that were "scratched" with a scalpel and coated with a coating mixture (FNC) (FNC contains bovine Fibronectin, Collagen (Type 1), and bovine serum albumin in HBS). The tumor fragments were placed on the "scratched" surface approximately 1 cm apart. A small volume of growth medium was added so that the entire surface of the dish was covered without immersing the tissue fragments in the medium. These explants were incubated at 37°C in a humidified incubator with a gas phase of 5% CO_2 in air. When substantial zones of cellular outgrowth were formed, the explanted tissues were transferred to another dish.

For both primary and subsequent subcultures (SC), cells were plated in the specific culture medium (as dictated by the experiment) and incubated at 37°C in an atmosphere of CO_2 in air . The medium was changed 3 times a week and monolayers were subcultured when they attained confluency. Anchorage-dependent cells were detached from the plastic by a dissociation medium containing Polyvinylpyrrolidone, Ethylene Glycol-bis (β-Aminoethyl Ether) N, N, N', N'-Tetraacetic Acid (EGTA), and Trypsin (PET), and the tryptic activity was inhibited by addition of Fetal Bovine Serum (FBS)-supplemented HBS. The cells were centrifuged and resuspended into the desired growth medium and a known inoculum size was then plated on FNC-coated plastic. The number of cells obtained at the subculture were recorded and used to prepare the Cumulative Population Doubling Levels (CPDL).

It was of prime importance that the cell lines be derived from the particular breast sample and not cross-contaminated by another specimen. Precautions against cross-contamination included cleaning the work surfaces with alcohol and irradiation of the Biohazard Hoods with ultraviolet light before handling each cell type, working on only one cell type at a time, and using a separate bottle of medium for each cell strain.

Description of various media. During the course of this grant, we used different culture media based on the previous studies reported by other investigators (19,37) and by our laboratory (13,34,65). Different "basal media" (37,39,56) were supplemented with a number of growth factors to produce serum-free media (18,34,66). Some of these Test Media were supplemented with added FBS at different concentrations. Based on our initial studies, we established three media during the grant period - BM0, BM2, and BM3. BM0 and BM2 were found to be best for the growth of normal breast cell lines and cancer breast cell lines, respectively. The differences between these media are summarized below. The complete composition for BRFF-BM0 and BRFF-BM2 is given in Tables A and B on Appendix Pages 33-34.

	BRFF-BM0	**BRFF-BM2**	**BRFF-BM3**
Growth Factors and Hormones			
β-estradiol	0	1.00E-08	1.00E-08
Prolactin	0	1 μg/ml	1 μg/ml
Dihydrotestosterone	0	1.00E-10	0
EGF	5 *n*g/ml	5 *n*g/ml	5 *n*g/ml
Insulin	5 μg/ml	5 μg/ml	5 μg/ml
Phosphoethanolamine	5.00E-06	5.00E-06	5.00E-06
Hydrocortisone	2.80E-07	2.80E-07	2.80E-07
Soy Bean Trypsin Inhibitor	10 μg/ml	10 μg/ml	10 μg/ml
Bovine Pituitary Extract	40 μg/ml	40 μg/ml	40 μg/ml

Cryopreservation of the breast cells. The cell suspensions were centrifuged and the pellets resuspended at a density of 4-6 x 10^6 cells per ml in ice-cold L-15 medium containing 10% FBS and 2x Gentamicin (100 mg/ml). An equal volume of ice-cold L-15 medium containing 10% FBS and 15% DMSO was added slowly (36) to the cell suspension. One ml aliquots of the cell suspension were transferred to pre-cooled cryovials, frozen slowly at a controlled rate and transferred to the vapor phase of a liquid nitrogen freezer. Samples of fresh breast tissue were also frozen and maintained at -80°C for possible future studies after establishing cell lines.

Transfection Experiments for Immortalization. The newly developed cell strains or explant cultures were transfected with pRSV-T DNA (17) using a lipofection method. The pRSV-T plasmid is an SV40 ori- construct containing the SV40 early region genes and the Rous sarcoma virus long terminal repeat (17,57). For each transfection, 5 μg DNA in 100 μl was mixed with an equal volume of diluted lipofectin or lipofectamine reagent (1:4 dilution) in a polystyrene tube and kept at room temperature for 15 min. It was then diluted to 2 ml with the culture medium. The monolayers were rinsed twice with the culture medium and then 2 ml of the DNA mixture was added drop-wise while gently swirling the dish. After 6-8 hr of incubation at 37°C, the medium was removed and replaced with regular medium and the transfected cultures were maintained using the routine conditions described above.

Transfection Experiments for Malignant Transformation. Transfections were carried out essentially as described above. The plasmid used in these experiments contains the mutated c-Ha-ras-1 oncogene from the T24 human bladder carcinoma and was provided by Dr. Premkumar Reddy, Fels Research Institute (58). Such cultures were subcultured at regular intervals with a view to generate new cell lines.

Morphological Studies. The cell cultures were routinely checked under a Nikon phase contrast inverted microscope and photographed. Scanned images of the photographs were captured on computer disks and printed using a laser printer.

Cytogenetic analysis and DNA fingerprinting. Semi-confluent cultures were sent to the Cell Culture Laboratory at Children's Hospital of Michigan for karyotypic analyses. Exponentially growing cultures were treated with 0.04 μg/ml of Colcemid for 1-2 hours, trypsinized and treated with 0.0375 M KCI for 9 minutes, and fixed in 3:1 methanol:glacial acetic acid mixture. The suspension was centrifuged and washed a couple of times with fixative and finally dropped on cold wet slides as previously reported (55) Slides were air dried and stained with 4% Giemsa solution and used for the determination of ploidy distribution, chromosome counts and constitutional aberrations. For trypsin Giemsa banding (GTG), karyotypes were prepared by a modified procedure of Seabright (61). The slides were aged at 60°C on a slide warmer for 16-20 hours, immersed in 0.025% trypsin for 1-2 seconds, stained with 4% Giemsa solution for 11 minutes, washed in buffer, dried and mounted in Permount. Well banded metaphases were photographed at 800x magnification using technical Pan film 2415 (Kodak) and printed on Rapidoprint FP 1-2 (Agfa-Geavert) or were karyotyped using the AKSII image analysis system. A minimum of 5 karyotypes were prepared from these prints and arranged according to standard karyotype (48). Genomic DNA samples isolated from different cell lines and the original breast tissue were used for DNA fingerprinting (76). The number of repeats of each of six different fragment length polymorphism (FLP) loci was determined.

Genomic DNA Isolation and PCR Amplification. Genomic DNA was isolated from the cell pellets of different cell lines following an established protocol which utilizes proteinase K digestion of the cells, phenol-chloroform extraction, and ethanol precipitation (67). The quantity and purity of the genomic DNA was then checked using a Pharmacia GeneQuant DNA calculator.

PCR amplification of the genomic DNA samples was performed in order to verify that the cells had been immortalized with the pRSV-T plasmid. One µg of purified DNA was utilized in PCR reactions. PCR amplification was performed using published amplimer sequences (see Table C on Appendix Page 35) and PCR conditions (17). Identification of the amplified gene was verified by loading 20 µl of the PCR reaction onto an agarose gel and performing electrophoresis.

PCR amplification of the genomic DNA samples was performed in order to characterize cell lines with respect to the presence or absence of specific proto-oncogenes and tumor suppressor genes. One µg of the genomic DNA was used in PCR reactions. PCR amplification of codons 12 and 61 of the *H-ras* oncogene was performed using Clontech's amplimer sets and the PCR protocol followed Clontech's suggested procedure. PCR amplification of the p53 tumor suppressor gene was

also performed using Clontech's amplimer sets and suggested protocol. Specifically, exon 8 of the p53 gene was amplified. Identification of the amplified genes was verified by loading 20 μl of each PCR reaction onto an agarose gel and performing electrophoresis.

RNA Isolation and Reverse Transcriptase-PCR (RT-PCR). RNA was isolated from human breast cells using the TRIzol reagent from Gibco/BRL and the recommended Gibco/BRL protocol.

For RT-PCR reactions, rTth polymerase from Perkin-Elmer was utilized. RT-PCR was performed using published primer sequences (see Table D on Appendix Page 36). RT-PCR conditions were determined empirically. RT-PCR conditions for cytokeratin 8 and cytokeratin 18 were as follows: RT reaction of 1 cycle of 60°C-30 minutes, 4°C-5 minutes and PCR reaction of 94°C-5 minutes; 30 cycles of 94°C-1 minute denaturation, 52°C-1 minute annealing, 72°C-2 minutes extension; 72°C-5 minutes. RT-PCR conditions for BRCA-1, erbB2, and Epidermal Growth Factor Receptor were as follows: RT reaction of 1 cycle of 60°C-30 minutes, 4°C-5 minutes and PCR reaction of 94°C-5 minutes; 40 cycles of 94°C-2 minutes denaturation, 52°C-2 minutes annealing, 72°C-2 minutes extension; 72°C-5 minutes. Identification of the amplified gene was verified by loading 20 μl of the RT-PCR reaction onto an agarose gel and performing electrophoresis.

Preliminary In Vitro Carcinogenesis Experiments: A focus assay was used in the preliminary *in vitro* chemical carcinogenesis experiment with 7,12-dimethyl benz(a)-anthracene (DMBA; 10 ng/ml), 20-methylcholanthrene (MCA; 100 ng/ml), and N-methyl-N^1-nitro-N-nitrosoguanidine (MNNG; 1 μg/ml). BRF97TA cells at SC 14 were seeded in the serum-free BM0 in FNC-coated dishes at 6 x 10^4 cells per 60 mm dish or the cells were seeded in BM0 supplemented with 5% FBS in non-FNC coated dishes. Twenty-four hours after plating, the cells were treated with carcinogens and maintained in culture for a maximum of 2.5 weeks. The dishes were rinsed with Phosphate Buffered Saline (PBS), then rinsed with PBS/10% methanol, fixed with 100% methanol for 15 minutes, and stained with 10% Giemsa in HBS. Dished containing "piled up" foci were photographed using an Ambis Image Analyzer. Live (unfixed) "piled up" foci from MNNG-treated dishes were also subcultured at regular intervals in order to generate additional cell lines.

Preliminary Study on the Effect of Anticancer Agents on Breast Cancer Cell Lines: A microassay in 96-well plates was employed using Sulforhodamine B (SRB) staining as the endpoint to test the effects of Paclitaxel (Taxol) and 5-fluorouracil (5-FU) on a breast cancer cell line. The SRB Assay described by Skehan *et al.* (63) was used after minor modifications to determine the SRB OD Units of cells grown under high density conditions. The plating of cells and drug treatment of the cells are similar to the published conditions. After 7 days in culture, the cells were fixed directly with cold TCA (50%) for 60 minutes, rinsed 5 times with tap water, and stained with 0.4% Sulforhodamine B (w/v) for 15 minutes. This was followed by rinsing the stained cells 5 times with 1% acetic acid. The plates were air-dried completely and examined under an inverted microscope for an overall qualitative examination. The dye from dried cells was solubilized with 200 μl of 10 mM Tris base (pH 10.5) for 5 minutes on a gyrotory shaker. The optical density of each well was read on a Microplate Reader (MR 600; Dynatech, Chantilly, Virginia) using a wavelength of 550 nanometers. The OD readings from the MR 600 plate reader were acquired through a capture program into an ASCII file which in turn was imported into a LOTUS worksheet for analysis.

Indirect Immunofluorescent Detection of Human Milk Fat Globulin (HMFG): Cells were grown on fluorescent microslides (Becton-Dickinson, Franklin Lakes, NJ) which were coated with FNC. After 24 hours, the slides were rinsed with PBS, fixed with cold acetone for 10 min, and air dried. The cells were rehydrated by incubating with PBS for 5 min and then incubated with 1:100 dilution of the IgG fraction of a rabbit polyclonal antibody against HMFG (Cat. AHP216, Serotec Limited, Oxford, England) for 60 min at room temperature in a moist chamber. The slides were then rinsed 3 times with PBS and incubated with 1:100 dilution of fluorescein-labeled goat anti rabbit IgG (Sigma) for 60 min. The slides were rinsed 5 times with PBS, mounted with a coverslip and examined under a Nikon fluorescence microscope. Epifluorescence (450-490 nm excitation filter and 520 nm barrier filter) was used to observe and photograph the cells.

Growth in Soft Agarose: First, a 0.75% nutrient agarose was prepared by mixing equal volumes of 1.5% Seaplaque agarose (FMC, Rockland, ME) and a 2x SFM, P4/8F medium (18), supplemented with 10% FBS and 0.05 mg/ml gentamycin. Next, a 4 ml base layer of 0.75% nutrient agarose was poured into 60 mm Petri dishes and allowed to solidify in the refrigerator. The 1 ml top layer consisted of 0.5 ml of 0.75% nutrient agarose mixed with 0.5 ml of cell suspension in the appropriate SFM. Therefore, the top layer in each dish contained a final concentration of 0.375% agarose, 2.5% FBS, and 10^4 to 10^5 cells. After 1 week of incubation, 1 ml of SFM was added over the top layer. After 10-12 days of total incubation, the dishes were stained with 0.1% tetrazolium solution in PBS (MTT, Sigma) for 1 hour. Stained three-dimensional colonies were counted under a stereomicroscope.

Tumorigenicity in nude mice: An equal number of both female and male athymic NCr-nu/nu nude mice were obtained and maintained in the animal room using Standard Operating Procedures operative at BRFF. Each cage housed 4 or 5 mice of the same sex.

The cells to be injected were isolated at the desired subculture by trypsinization. Tryptic activity was then inactivated by addition of HEPES buffered saline (HBS) containing 5% FBS. The cell suspension was centrifuged, the cell pellet resuspended in HBS, and the cell number determined. The cell suspension was again centrifuged and the cell pellet resuspended in HBS to make the concentrated cell suspension which was then injected into the mice. Five to 10 million cells were injected subcutaneously. In some cases, the cells were also injected intraperitoneally.

The mice were checked at regular intervals for the presence of tumors.

Results and Discussion

This grant involved multiple functions which were interdependent. The establishment of cell cultures required appropriate serum-free media. However, the appropriate serum-free media could only be tested using various cell strains and cell lines. Therefore, the different functions described below often were performed concurrently. We have discussed each function separately in order to facilitate understanding.

1. Establishment of Serum-Free Media for Breast Cell Cultures

The first step was to preferentially grow breast epithelial cells whose proliferation may be regulated not only by growth factors but also by hormones. Serum-supplemented culture media are known to selectively grow fibroblasts from explant cultures and from primary cells isolated from tissue fragments. Indeed, a fibroblastic cell line, BRF109, was generated when explant tissue was maintained in a classical basal medium, DME/F12(3) supplemented with FBS. In order to develop and maintain breast epithelial cell cultures, a variety of serum-free culture media (SFM) fortified with appropriate growth factors and hormone combinations were tested for their ability to produce outgrowths of epithelial cells from the tissue explants. Two such media, BRFF-BM0 and BRFF-BM2, which generated good epithelial cell growth of the primary cultures were used for the routine maintenance of the cell cultures (Table A and B in the Appendix). These media differ with regards to the presence of β-estradiol, prolactin, and dihydrotestosterone. BRFF-BM0 medium produced better epithelial cellular outgrowths from explants of normal breast tissue and BRFF-BM2 medium produced better epithelial cellular outgrowths from breast cancer tissue explants.

In an effort to develop even better media for the breast cell cultures, we made two new basal media . These media were made by manipulating the concentration of each component from both HPC-1 basal (which is the basal medium used for BRFF-BM0 and BRFF-BM2) and another medium used for breast cell culture called MCDB-170 basal. For the first new basal medium (PT1 basal), the higher concentration of each component present in HPC-1 basal or MCDB-170 basal was used. For the second basal medium (PT2 basal), the average concentration from the two basal media was used. We checked PT1 basal and PT2 basal supplemented with each of the hormones and growth factors which had been used previously. Our results showed that use of PT1 basal or PT2 basal did not increase the quality or growth of the breast cell cultures.

Therefore, we continued to use BRFF-BM0 and BRFF-BM2 for culture maintenance. We have found that BRFF-BM0 is best used for the initial explant cultures in order to obtain good cellular outgrowth. Cultures from normal tissues will continue to grow well in BRFF-BM0. Cultures from some cancer tissues also will grow well in BRFF-BM0; however, cultures from other cancer tissues may require BRFF-BM2. In some cases, certain cancer cultures may require specialized hormone combinations (e.g., BRFF-BM3) or growth factors.

We continued to test various growth factors and hormones to see if they would enhance the growth of breast cell cultures. The effect of Insulin-like Growth factor (IGF)(14,33) and basic Fibroblastic Growth Factor (bFGF) (15,68) on the growth of cells derived from cancer tissues was tested. The effect of the above factors was checked in both BRFF-BM0 and in BRFF-BM2. For

these cells, the proliferative capacity was increased by bFGF but not by IGF. Additional studies will be required to determine the optimal concentration of bFGF for a particular cell type.

2. Description of the New Human Breast Tissue Obtained

During the grant, we received a total of 68 tissue samples mainly from Ohio State University. Fourteen tissues were received in a cryopreserved state but 12 of these were not ideal for cell culture development. In addition, one tissue sample was contaminated with yeast and had to be discarded. Of the 55 viable tissue samples, 3 were from reductive mammoplasty of normal subjects and 52 were from cancer patients. The 52 samples derived from the cancer patients included 24 carcinomas, 16 metastases (primarily into lymph node), and 12 uninvolved normal areas. Background of the viable tissues are listed in Table E on Appendix Pages 37-38.

If sufficient amounts of tissue were available, a small portion was frozen for DNA profile comparison after a cell line was established. If relatively large amounts of tissue were available, a portion was cut into small pieces and cryopreserved in a modified L15 freezing medium.

All samples received were entered into the BRFF breast tissue database. Information for each tissue sample included: Date of Tissue Receipt; Origin of Sample - Person, City, State; Supplier's Tissue ID; BRFF Tissue ID; Patient Information - Sex, Age, Race; Date of Tissue Removal from Patient; Location of Tissue in the Body; Background of Tissue; Pathology; Pathology Report Received or Not; Weight of Tissue; Medium used for Tissue Transport; Comments; Storage Location at BRFF.

3. Establishment of New Breast Cell Cultures

During the course of this grant, we developed many cell cultures from tissue samples. Most of the explanted tissues produced cellular outgrowths. However, many of the outgrowths could not be subcultured. A list of all cultures which completed the first subculture is given in Table F on Appendix Pages 39-42.

BRFF cell ID numbers are derived from BRFF tissue numbers (e.g., BRF109 is derived from BRFF109 tissue). Therefore, the background for a particular cell line is easily associated with the original tissue from which the cell line was derived. When isolated cell suspensions or explant cultures were transfected with pRSV-T, these transfected cell cultures are identified with a capital T after the BRF cell number. If a cell line was derived from a T24 plasmid transfection (ras oncogene), then a capital R is after the BRF cell number. Any cell line established from different experimental conditions is identified by using A,B,C, etc. Please note that SC denotes subculture or passage number.

Table 1 (on the next page) summarizes the salient features of the breast cell strains/lines that were developed at BRFF during the grant period. A cell strain is one that passed SC1 and could be cryopreserved whereas a cell line is a culture that progressed past SC10. During this grant, 30 cultures had undergone a second subculture and were expanded to a sufficient number for cryopreservation. Of these 30, 20 are classified as cell strains. (We have already depleted 3 of these cell strains to develop appropriate media and to test growth conditions.)

Table 1. List of Viable Breast Cell Strains/Cell Lines at BRFF. **(Bold type indicates a cell line)**

Cell ID	Start Date	Transfected with Plasmid	SC1 Date	SC5 Date	SC10 Date	Comments	Morphology
BRF029	**10/05/1994**		**10/17/1994**	**12/06/1994**	**01/10/1995**	**6 vials cryopreserved**	**Fibroblastic**
BRF029TA	10/05/1994	pRSVT	10/21/1994			1 vial cryopreserved	
BRF029TB	10/05/1994	pRSVT	10/21/1994			1 vial cryopreserved	
BRF036	10/26/1994		11/17/1994			Used for testing	
BRF047TI	01/24/1995	pRSVT	03/13/1995	05/15/1995		Used for testing	
BRF049A	02/03/1995		02/17/1995			Used for testing	
BRF069TC	**04/04/1995**	**pRSVT**	**05/23/1995**	**07/03/1995**	**08/25/1995**	**20 vials cryopreserved**	**Epithelial**
BRF071TB	**04/20/1995**	**pRSVT**	**05/16/1995**	**06/13/1995**	**07/17/1995**	**54 vials cryopreserved**	**Epithelial**
BRF071TC	**04/20/1995**	**pRSVT**	**05/16/1995**	**06/16/1995**	**07/17/1995**	**18 vials cryopreserved**	**Epithelial**
BRF071TR	**04/20/1995**	**T24 DNA**	**05/16/1995**	**06/16/1995**	**04/04/1996**	**2 vials cryopreserved**	**Epithelial**
BRF079TA	07/21/1995	pRSVT	08/14/1995	09/26/1995		1 vial cryopreserved	
BRF079TB	07/21/1995	pRSVT	08/16/1995			1 vial cryopreserved	
BRF082TB	**08/11/1995**	**pRSVT**	**08/28/1995**	**10/05/1995**	**11/16/1995**	**8 vials cryopreserved**	**Epithelial**
BRF087	08/31/1995		09/11/1995			2 vials cryopreserved	
BRF088A	08/31/1995		09/11/1995			3 vials cryopreserved	
BRF088TB	08/31/1995	pRSVT	09/11/1995	11/01/1995		2 vials cryopreserved	
BRF089	08/31/1995		10/02/1995	11/02/1995		6 vials cryopreserved	
BRF096B	09/13/1995		10/19/1995			1 vial cryopreserved	
BRF097TA	**09/15/1995**	**pRSVT**	**10/12/1995**	**12/27/1995**	**02/15/1996**	**34 vials cryopreserved**	**Epithelial**
BRF097TN	**03/11/1996**	**MNNG**	**03/29/1996**	**05/01/1996**	**06/06/1996**	**27 vials cryopreserved**	**Epithelial**
BRF097TR	**09/15/1995**	**T24 DNA**	**10/12/1995**	**12/27/1995**	**02/15/1996**	**13 vials cryopreserved**	**Epithelial**
BRF098R	09/15/1995	T24 DNA	10/23/1995	12/14/1995		9 vials cryopreserved	
BRF099R	09/15/1995	T24 DNA	10/23/1995	12/26/1995		3 vials cryopreserved	
BRF105TB	03/05/1996	pRSVT	06/27/1996			2 vials cryopreserved	
BRF109	**03/27/1996**		**04/26/1996**	**05/28/1996**	**07/03/1996**	**5 vials cryopreserved**	**Fibroblastic**
BRF110TB	05/09/1996	pRSVT	05/28/1996	07/03/1996		1 vial cryopreserved	
BRF117	08/01/1996		08/27/1996			1 vial cryopreserved	
BRF129	11/07/1996		12/02/1996			1 vial cryopreserved	
BRF130	11/26/1996		12/31/1996			1 vial cryopreserved	
BRF135T	03/20/1997	pRSVT	04/10/1997	05/29/1997		10 vials cryopreserved	

The 10 remaining cultures are cell lines. BRFF has developed 8 immortal breast epithelial cell lines (BRF69TC, BRF71TB, BRF71TC, BRF71TR, BRF82TB, BRF97TA, BRF97TN, BRF97TR) and 2 established fibroblastic cell lines without immortalization (BRF29, BRF109).

3a. Description of cell cultures derived from breast tissue

This first approach was primarily used to generate cell cultures. We utilized tissue explants to generate cellular outgrowths which were then transfected with an immortalizing gene to generate cell strains and cell lines. Photographs of a typical explant culture and cellular outgrowth are shown in Figs. 1 and 2 on Appendix Page 53, respectively.

It is important to note that primary cells isolated by enzymatic digestion using collagenase and trypsin (8) did not yield any subculturable monolayer cultures. In addition, cryopreserved breast tissues rarely generated epithelial cell strains.

We generated many cell strains (Table F in the Appendix). Some of these strains became contaminated and had to be discarded whereas others had to be abandoned because of senescence.

We were able to generate many cell strains from explants without transfection with an immortalizing gene. We cryopreserved 9 of these untransfected epithelial cell strains of which 7 may be utilized in the future (Table 1). In addition, we developed two untransfected breast fibroblastic cell lines which could be grown for over 10 SC without any sign of senescence. These fibroblastic lines, BRF29 and BRF109, grow in the presence of 5% Fetal Bovine Serum. In the future, these cell lines may be useful for studying the interaction of stromal and epithelial breast cells.

As in other types of human cells, the breast epithelial cells exhibited senescence after a limited number of cell divisions. To make permanent epithelial cell lines from the epithelial cell strains, we chose to immortalize the cells with the pRSV-T plasmid which contains the SV-40 early region T-antigen gene.(17,57) We generated 14 cell strains that were transfected with the pRSV-T plasmid. Of these strains, 3 were derived from primary breast cancer (BRF105TB, BRF110TB, and BRF135T). The most successful primary breast cancer strain was BRF135T. The strain underwent several SC and has been cryopreserved for future use.

Five established cell lines grew out of some of the transfected cell strains (BRF69TC, BRF71TB, BRF71TC, BRF82TB, and BRF97TA). All of these except BRF82TB have been in continuous culture for a minimum of 20 weeks.

Three cell lines were developed from metastases to the lymph node (BRFF69TC, BRF71TB, and BRF71TC). The BRF69TC cell line was derived from a lymph node metastasis of breast cancer. After 35 days in culture, the explants along with the cellular outgrowth were transfected with the pRSV-T plasmid. The cell line was cultured continually for 11 subcultures. Fig. 3 on Appendix Page 53 shows a photograph of BRF69TC at SC4. The BRF71TB and BRF71TC cell lines were developed from lymph node metastasis of breast cancer. The two cell lines were essentially identical except they were derived from two separate transfections. BRF71TB was developed from an explant dish transfected on day 12 with the pRSV-T plasmid. BRF71TB was cultured continually

for 37 subcultures and cryopreserved. Fig. 4 on Appendix Page 54 shows a photograph of BRF71TB at SC35. BRF71TC was developed from another explant dish which was transfected on day 19 with the pRSV-T plasmid. BRF71TC was cultured continually for 16 subcultures and cryopreserved. Fig. 5 on Appendix Page 54 shows a photograph of BRF71TC at SC6. All three cell lines exhibited a predominantly epithelial morphology.

We established two new normal cell lines - BRF82TB and BRF97TA. The BRF82TB cell line was derived from a reductive mammoplasty tissue sample from a 27 year old Black female patient. The cells were isolated by enzymatic treatment of minced tissue. The cells were plated in an FNC-Coated T-25 vented flask and fed BRFF-BM0. After 13 days in the flask, the cells were transfected using pRSV-T and Lipofectamine. The cells have been subcultured to Subculture 11 and have undergone 8 population doublings in 109 days. The BRF82TB cell line was not used for future studies because of an extremely abnormal chromosomal profile. A photograph showing BRF82TB at SC 10 is given in Fig. 6 on Appendix Page 54.

The BRF97TA cell line was derived from a reductive mammoplasty tissue sample from a 39 year old Caucasian female patient. To generate BRF97TA, cells were isolated from mechanical dissociation of minced tissue. The cells were plated in an FNC-Coated T-25 vented flask and maintained with BM0. After 25 days in the flask, the cells were transfected with the pRSV-T plasmid. The cells were subcultured two days later and seeded into 2 x T-25 FNC-Coated flasks. BRF97TA was cultured continually for 25 subcultures and cryopreserved. Photographs showing BRF97TA at SC 1 and 16 are given in Figs. 7a and 7b, respectively on Appendix Page 55. A distinct epithelial morphology is evident.

3b. Description of breast cell cultures established by transfection with H-ras oncogene

A second approach to establish malignant breast cell cultures was tried. The strategy was to transfect cells derived from cellular outgrowth of explants of normal breast tissue with the T24 plasmid which contains the activated (mutated) H-ras oncogene. This approach yielded two cell strains, BRF98R and BRF99R, and two cell lines, BRF97TR and BRF71TR.

The BRF98R cell strain was derived from a reductive mammoplasty tissue sample from a 34 year old Caucasian female patient. The cell strain was derived from an explant culture and the cellular outgrowth was isolated and transfected with the T24 plasmid. The BRF98R cell strain was cultured continually for 6 subcultures.

The BRF99R cell strain was derived from uninvolved normal breast tissue from a cancer patient (53 year old Caucasian female patient). The cell strain was derived from an explant culture and the cellular outgrowth was isolated and transfected with the T24 plasmid. The BRF99R cell strain was cultured continually for 7 subcultures. A photograph of BRF99R at SC3 is shown in Figure 8 on Appendix Page 55.

We also transfected an immortalized normal breast cell line (BRF97TA) at SC11 with the T24 plasmid to yield a new malignantly transformed cell line, BRF97TR. After transfection with the T24 plasmid this culture went through an additional 14 subcultures (ie, cumulative SC25 after

the initiation of BRF97TA). A photograph of BRF97TR at SC23 is shown in Figure 9 on Appendix Page 56.

In addition, a cell line developed from a metastatic cancer tissue after immortalization with pRSV-T (BRF71TC) was retransfected at SC 5 with the T24 plasmid to develop BRF71TR. The population doubling for this cell line was 37 in 102 days.

3c. Description of breast cell cultures established by treatment with mutagenic/carcinogenic chemicals.

A third approach to establish malignant breast cell cultures was to treat an immortalized normal breast cell line (BRF97TA) with a chemical carcinogen. We tested three different mutagenic/carcinogenic chemicals and the most effective chemical was MNNG. After treatment with MNNG, considerable cell death was seen in the dishes. However, foci of fast growing cells evolved from such treated cultures (Fig. 11 on Appendix Page 57). Some of the control as well as the MNNG-treated cells were fixed and stained to show the dramatic effects of MNNG treatment (Fig. 12 on Appendix Page 58). There were many more "piled up" foci in dishes grown in medium that did not contain any FBS . The formation of piled up colonies is generally regarded as the manifestation of a transformed phenotype. The foci of fast growing cells were used to establish the BRF97TN cell line. This cell line was cultured continually for 11 additional SC (*i.e.*, cumulative SC 25 after initiation of BRF97TA) and then cryopreserved. A photograph of BRF97TN at SC24 is shown in Figure 10 on Appendix Page 56.

4. Characterization of Established Breast Cell Lines

The cell strains were not characterized because they had not gone past an SC level that would be useful to investigators.

The cell lines, BRF29 and BRF109, were not subjected to detailed characterization because they are fibroblastic cell lines and our main emphasis was on epithelial cell lines. We did document the population doubling for BRF29 which was 52 in 87 days and the population doubling for BRF109 which was 15 in 90 days.

BRF82TB, a normal breast epithelial cell line derived from reductive mammoplasty, had an extremely abnormal chromosomal profile and was therefore, not studied further. The population doubling for this cell line was 8 in 97 days.

In the following sections, we describe the characterization of BRF69TC, BRF71TB, BRF71TC, BRF97TA, BRF97TN, and BRF97TR. All of these established cell lines were found to be free of mycoplasma contamination by the ATCC. The following characteristics and assays for the established cell lines were determined: morphology, Cumulative Population Doubling Levels (CPDL), chromosome analysis/DNA fingerprinting, DNA/RNA analysis, growth in soft agarose, and tumorigenic potential in nude mice.

Cumulative Population Doubling Levels

CPDL of BRF69TC, BRF71TB, and BRF71TC are given in Fig. 13a on Appendix Page 59. Each point on the graph represents a subculture. The cancer cell line, BRF69TC, underwent 38 population doublings in 169 days. The cancer cell lines derived from the same tissue, BRF71TB and BRF71TC, had similar cell proliferation patterns as can be seen by the overlapping plots. The BRF71TB cell line underwent 135 population doublings in 372 days. BRF71TC underwent 59 population doublings in 138 days.

CPDL of BRF97TA, BRF97TR, and BRF97TN are given in Fig. 13b on Appendix Page 59. The normal cell line, BRF97TA, underwent an initial slow growth phase. There was an 8 week period before SC2 was possible. However, after this time the cells grew rapidly and the cell line underwent 92 population doublings in 245 days. BRF97TR and BRF97TN, the transformed derivatives of BRF97TA, underwent 96 population doublings in 243 days and 88 population doublings in 257 days, respectively.

Due to their continuous culture for at least 150 days, the above six cell lines can be regarded as permanent cell lines. Various subcultures for these different cell lines were cryopreserved and stored in the BRFF Cell Repository (see below).

Chromosome Analysis

Karyotypic analysis of BRF69TC(at SC4), BRF71TB (at SC5 and SC38), BRF71TC (at SC5), BRF97TA (at SC6 and SC25), BRF97TR (at SC25), and BRF97TN (at SC25) were performed. Male Y chromosome was not present in any of the cell lines after QM staining. None of these cell lines were contaminated with other cell lines. Results of ploidy distribution studies from 100 metaphases showed that most cell lines had a chromosome count in the diploid range. All cell lines except the normal breast cell line at early SC (BRF97TA at SC6) contained various marker chromosomes. Karyotypes and additional details for the above cell lines are given in Figs. 14 - 19 on Appendix Pages 60-67.

DNA Fingerprinting

BRF69TC, BRF71TB, BRF71TC, BRF97TA, BRF97TR, and BRF97TN were examined by DNA fingerprinting (76). The number of repeats of each of six different fragment length polymorphism (FLP) loci was determined. The new cell lines have fingerprints identical to the tissue used to establish the respective cell lines. BRF69TC, the BRF71T cell lines, and the BRF97T cell lines have distinctly different fingerprints. BRF71TB and BRF71TC share 5 out of 6 FLP indicating these cell lines are similar. In addition, BRF97TA and its derivatives, BRF97TR and BRF97TN, have identical DNA fingerprints.

DNA and RNA Analysis

PCR was performed for the SV40 T-antigen gene (pRSV-T) in order to see if a cell line was stably transfected and retained the SV40 T-antigen gene. In addition, PCR was used to elucidate the presence or absence of two proto-oncogenes, the H-*ras* and K-*ras* genes, and the p53 tumor suppressor gene. It is important to note that alterations of proto-oncogenes and tumor suppressor genes (29,32) play a key role in cancer progression, and mutated oncogenes and suppressor genes are present in mammary tumors (51,52,69,70). All samples generated the specific PCR products indicating that the cell lines contain SV40 T-antigen gene, H-ras gene, K-ras gene, and the p53 tumor suppressor gene (Fig. 20 on Appendix Page 68). These results are summarized in Table 2 below.

Reverse Transcriptase PCR (RT-PCR) was used to determine the expression of the following genes; cytokeratin 8, cytokeratin 18, erbB2, epidermal growth factor receptor (EGFR), and BRCA-1. The rationale for selecting these genes were: cytokeratin 8 and cytokeratin 18 have been shown to be markers for epithelial cells (38,40,47); BRCA-1 and erbB2 have been suggested as markers for breast cancer (4,6,10,12,18,23,49,50,53,59,62); EGFR may be a prognostic marker for breast cancer (59,71). All of the cell lines expressed all of the above markers (Fig. 21 on Appendix Page 69). These results are summarized in Table 2 below.

Table 2. Results from PCR and RT-PCR Experiments

A + sign indicates that all experiments did produce amplified DNA bands

Cell Line	**Genes for**				**Expression of RNA for**				
	SV40 T-antigen (pRSVT)	H-ras	K-ras	p53	Cytokeratin 8	Cytokeratin 18	BRCA-1	erb-B2	EGFR
BRF69TC	+	+	+	+	+	+	+	+	+
BRF71TB	+	+	+	+	+	+	+	+	+
BRF71TC	+	+	+	+	+	+	+	+	+
BRF97TA	+	+	+	+	+	+	+ *	+	+ *

*RT-PCR experiments were repeated 3-5 times. For BRF97TA, BRCA-1 and EGFR showed amplified DNA bands in 3 out of 4 experiments.

Detection of Human Milk Fat Globulin (HMFG)

In order to determine if the newly developed human breast cell lines exhibited differentiated breast functions, we studied the production of HMFG in BRF71TB and BRF97TA. Both cell lines produced HMFG as shown in Fig.22a and 22b on Appendix Page 70. The fluorescence was localized exclusively in the cytoplasm.

Growth in Soft Agarose

Cancer cells have the capacity to grow as three-dimensional colonies in soft agarose. Such anchorage independent growth is not seen in normal cells. We observed that BRF69TC, BRF71TB, and BRF71TC exhibited anchorage independent growth with varying degrees of colony forming efficiency. BRF71TB produced a small number of colonies when 1×10^4 cells were plated in the top layer; however, if the number of cells in the top layer was increased to 4×10^4 then a colony forming efficiency of 2.06% was observed. For all three lines, if the number of cells plated was further increased, the colonies formed were too numerous to count.

The normal cell line, BRF97TA, at an early subculture (SC6) did not produce colonies under the same experimental conditions. However, BRF97TA at late subcultures generated a colony forming efficiency of 0.29%. Therefore, this normal cell line may have acquired some malignant properties at late SC levels.

The BRF97TR and BRF97TN cell lines (transformed derivatives of BRF97TA) exhibited a greater capacity for anchorage independent growth as compared to the mother culture, BRF97TA.

Tumorigenicity in Nude Mice

The tumorigenic potential of BRF69TC, BRF71TB, BRF97TA, BRF97TR, and BRF97TN was evaluated (Table G on Appendix Page 43). Both early and late passages of BRF97TA were included. In addition, a positive control cell line, MCA-20 P+2, known to produce tumors in nude mice also was used.

The positive control cell line developed tumors in 4/4 female mice and 4/4 male mice within 1 week after injection and the mice were sacrificed on Day 10. No tumors were developed from any of the breast cells injected into either female or male mice for the duration of study (116 -156 days). Therefore, the cell lines established in this study do not possess tumorigenic potential in nude mice.

5. Potential Use of the Breast Cancer Cell Lines

In order to test if the established breast cancer cell lines would be useful in the identification and testing of new anticancer agents, we performed a preliminary experiment with BRF71TC using two established anticancer agents, taxol and 5-fluorouracil (5-FU). Both these agents are used in the treatment of human breast cancer (26). The effects of taxol and 5-FU on BRF71TC are shown in

Figure 23a and 23b, respectively on Appendix Page 70. Both the agents showed significant cytotoxic effects on BRF71TC. However, to obtain 50% killing of BRF71TC, approximately 1×10^{-13} M of taxol was sufficient while 1×10^{-5} M of 5-FU was needed. The lower concentration of taxol indicates that it is a more effective agent than 5-FU. This preliminary experiment shows that the established cancer cell lines will be useful in screening anti-breast cancer agents.

6. Breast Cell Repository and DNA Bank at BRFF

During the course of this grant, we obtained a liquid nitrogen freezer for the purpose of storing the breast cell strains/lines. We have also set aside space in a mechanical freezer (-80°C) for the storage of DNA samples isolated from the breast cell lines. Therefore, the necessary infrastructure for the operation of the BRFF cell repository and DNA bank is in place. All managerial and administrative aspects of the BRFF repository for breast cancer cells and genomic DNA have been implemented. Computerized databases for both the cell repository and the DNA bank have been developed. Listings of the inventories for the cell repository and DNA bank are given in Tables H and I on Appendix Pages 44-49 and 50-52, respectively.

We have announced the availability of BRF71TB to the research community by placing an advertisement in Cancer Research and in Clinical Cancer Research (to be published in November, 1997). In addition, photographs of the available breast cell lines, BRF71TB and BRF97TA, can be seen on our web page (http://www.brff.com). Therefore, the repository and DNA bank have been opened to the research community.

Recommendations in Relation to the Statement of Work

In the Statement of Work we proposed to produce 25 new cell lines from human breast cancer. To date, we have developed 10 cell lines and 20 cell strains. It is possible that some of the cell strains may become cell lines if cultured continuously by other investigators. (We will make these cell strains available to other investigators.) It was difficult to generate new breast cell lines and we recommend that more research on this topic be performed with emphasis on developing cultures from primary breast cancers. In addition, we characterized specific properties of the cell lines as described in this report. However, further characterization of these cultures may be necessary. The cell repository and DNA bank have been implemented. In the future, we recommend that more cell cultures be developed and added to the repository. Generating cell cultures from non-Caucasian patients should be considered.

CONCLUSIONS

During the first year, we developed 10 cell cultures from human breast cancer tissues. Six of these cell cultures were classified as cell strains because they had not been in continuous culture for more than 10 subcultures . These cell strains were cryopreserved and some of the strains became cell lines in the following year.

During the second year, we researched information on breast cell lines (1,5-7,11,18,20,28,40,41,60) and the effect of various chemicals on breast and other epithelial cells (14,16,21,22,24,25,33,44) which had been published by many authors. We used this information in our characterization of the established cell lines and in the formulation of new serum-free culture media. During this period, we developed 11 cell strains and 6 cell lines. The new breast cell lines and cell strains were developed from breast cancer tissues, uninvolved tissues from cancer patients and normal tissues from reductive mammoplasty patients. Two different approaches were tried to generate breast cell lines with malignant properties: (a) transfecting the breast cells with a plasmid containing mutated *ras* oncogene and (b) treating a normal (non-malignant) breast cell with a mutagenic chemical *in vitro*. Some of the established cell lines were then partially characterized with respect to karyotype, the presence of the H-*ras* and p53 genes, expression of specific gene markers, and cumulative population doubling levels. We also performed a preliminary experiment to determine the effects of two anticancer agents, taxol and 5-fluorouracil on an established cancer cell line.

During the final year, we continued to characterize some of the established cell lines. We focused on generating cell strains/lines from primary breast tumors rather than from metastatic tumors. From the ten primary tissues received, five cell strains were developed. However, only one of these cell strains, BRF135T, was propagated for 5 subcultures, cryopreserved, and added to the cell repository. In addition, we developed two more cell strains from metastatic tumors. These strains were cryopreserved and added to the cell repository.

All the available cell strains/cell lines in the BRFF repository are shown in Table H on Appendix Pages 44-49. In addition, the genomic DNA isolated from the cell lines is stored in the DNA bank (Table I on Appendix Pages 50-52).

Details of the ten cell lines established at BRFF during this grant are summarized in Table 3 on the next page.

Table 3. Summary of Breast Cell Lines Established at BRFF

Name of Cell Line	Background	Morphology	Days in Culture	CPDL
BRF-69TC	Metastatic to Lymph Node	Epithelial	169	38
BRF-71TB	Metastatic to Lymph Node	Epithelial	372	135
BRF-71TC	Metastatic to Lymph Node	Epithelial	138	59
BRF-71TR	BRF-71TC transfected with H-ras oncogene	Epithelial	102	37
BRF-97TA	Normal tissue from reductive mammoplasty patient	Epithelial	245	92
BRF-97TR	BRF-97TA transfected with H-ras oncogene	Epithelial	243	96
BRF-97TN	BRF-97TA treated with MNNG	Epithelial	257	88
BRF-82TB	Normal tissue from reductive mammoplasty patient	Epithelial	97	8
BRF-29	Normal tissue from Cancer Patient	Fibroblastic	87	52
BRF-109	Cancer tissue from Cancer Patient	Fibroblastic	90	15

Summary of the Implication of the Completed Research

We have developed 10 cell lines and 20 cell strains which are available to the scientific community. In a preliminary experiment, we found that one of the new breast cancer cell lines may be a good target for screening anticancer agents. It is envisioned that the new breast cell lines described here will be useful as targets for the identification of therapeutic drugs against breast cancer and as models for carcinogenesis *in vitro*. In addition, the serum-free media and experimental conditions described in this study will be useful in establishing new normal and breast cancer epithelial cell lines in other laboratories.

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ADDITIONAL INFORMATION

1. Bibliography of Publications and Meeting Abstracts

We have communicated our research findings in two ways. First, we presented a poster at the American Association for Cancer Research (AACR) meeting in Washington, DC in April, 1996. An abstract of this poster is published in the Proceedings of AACR (see below). Second, a manuscript describing our results has recently been accepted by *Cytotechnology* (see below).

We will also be presenting a poster at the Breast Cancer Research Program: An Era of Hope in October, 1997.

List of Publications

Iype, P.T., Michael, M., Wojcik, M., Iype, L., and Verma, M. Establishment of immortalized human breast cell lines for studies in carcinogenesis and drug development. Proceedings of AACR, *37*: 114-115, 1996.(Abstract)

Iype, L.E., Michael, M., Verma, M, and Iype, P.T. Development and characterization of new immortalized human breast cell lines. Cytotechnology (In Press).

2. List of Personnel in this Effort

Dr. P. Thomas Iype
Dr. Lisa Iype
Dr. Mukesh Verma
Ms. Sarah Lebherz
Ms. Michele Michael
Ms. Andrea Wegley

Appendix

Table A. Composition of BRFF-BMZERO

COMPONENTS	CONCENTRATION *Moles/Liter*
ESSENTIAL AMINO ACIDS	
L-ARGININE	2.00E-03
L-CYSTINE	1.50E-04
L-HISTIDINE	2.00E-04
L-ISOLEUCINE	6.00E-05
L-LEUCINE	2.00E-04
L-LYSINE	4.00E-04
L-METHIONINE	6.00E-05
L-PHENYLALANINE	6.00E-05
L-THREONINE	2.00E-04
L-TRYPTOPHAN	2.00E-05
L-TYROSINE	6.00E-05
L-VALINE	2.00E-04
NONESSENTIAL AMINO ACIDS	
L-ALANINE	2.00E-04
L-ASPARAGINE	2.00E-04
L-ASPARTIC ACID	2.00E-04
L-GLUTAMIC ACID	2.00E-04
L-GLUTAMINE	2.00E-03
GLYCINE	2.00E-04
L-PROLINE	6.00E-04
L-SERINE	2.00E-04
VITAMINS	
d-BIOTIN	3.00E-07
FOLIC ACID	3.00E-06
DL-LIPOIC ACID	1.00E-06
NIACINAMIDE	3.00E-07
D-PANTOTHENATE (Calcium)	1.00E-06
PYRIDOXINE	3.00E-07
RIBOFLAVIN	1.00E-07
THIAMINE	1.00E-06
VITAMIN B_{12}	1.00E-06
BUFFERS	
HEPES	1.50E-02
SODIUM BICARBONATE	1.40E-02

COMPONENTS	CONCENTRATION *Moles/Liter*	
HORMONES/GROWTH FACTORS		
EPIDERMAL GROWTH FACTOR		5 *ng*/ml
BOVINE PITUITARY EXTRACT		40µg/ml
HYDROCORTISONE	2.80E-07	
INSULIN		5 µg/ml
OTHER ORGANICS		
BOVINE SERUM ALBUMIN		250µg/ml
CHOLERA TOXIN		25 *ng*/ml
CHOLINE CHLORIDE	1.00E-04	
D-GLUCOSE	7.00E-03	
HYPOXANTHINE	3.00E-05	
myo-INOSITOL	1.00E-04	
PHOSPHOETHANOLAMINE	5.00E-06	
PUTRESCINE	2.00E-06	
PYRUVIC ACID (Sodium)	2.00E-03	
THYMIDINE	3.00E-06	
TRYPSIN INHIBITOR		10 ug/ml
INORGANIC SALTS		
CALCIUM CHLORIDE	2.00E-04	
MAGNESIUM CHLORIDE	5.20E-04	
MAGNESIUM SULFATE	1.60E-04	
POTASSIUM CHLORIDE	3.80E-03	
POTASSIUM PHOSPHATE(Mono)	4.30E-04	
SODIUM CHLORIDE	1.12E-01	
SODIUM PHOSPHATE(Dibasic)	8.90E-04	
TRACE ELEMENTS		
CUPRIC SULFATE	1.00E-08	
FERROUS SULFATE	3.00E-06	
SELENIOUS ACID	3.00E-08	
ZINC SULFATE	5.00E-07	

Table B. Composition of BRFF-BM2

COMPONENTS	CONCENTRATION *Moles/Liter*
ESSENTIAL AMINO ACIDS	
L-ARGININE	2.00E-03
L-CYSTINE	1.50E-04
L-HISTIDINE	2.00E-04
L-ISOLEUCINE	6.00E-05
L-LEUCINE	2.00E-04
L-LYSINE	4.00E-04
L-METHIONINE	6.00E-05
L-PHENYLALANINE	6.00E-05
L-THREONINE	2.00E-04
L-TRYPTOPHAN	2.00E-05
L-TYROSINE	6.00E-05
L-VALINE	2.00E-04
NONESSENTIAL AMINO ACIDS	
L-ALANINE	2.00E-04
L-ASPARAGINE	2.00E-04
L-ASPARTIC ACID	2.00E-04
L-GLUTAMIC ACID	2.00E-04
L-GLUTAMINE	2.00E-03
GLYCINE	2.00E-04
L-PROLINE	6.00E-04
L-SERINE	2.00E-04
VITAMINS	
d-BIOTIN	3.00E-07
FOLIC ACID	3.00E-06
DL-LIPOIC ACID	1.00E-06
NIACINAMIDE	3.00E-07
D-PANTOTHENATE (Calcium)	1.00E-06
PYRIDOXINE	3.00E-07
RIBOFLAVIN	1.00E-07
THIAMINE	1.00E-06
VITAMIN B_{12}	1.00E-06
BUFFERS	
HEPES	1.50E-02
SODIUM BICARBONATE	1.40E-02

COMPONENTS	CONCENTRATION *Moles/Liter*
HORMONES/GROWTH FACTORS	
EPIDERMAL GROWTH FACTOR	5 *ng*/ml
BOVINE PITUITARY EXTRACT	40µg/ml
HYDROCORTISONE	2.80E-07
INSULIN	5 µg/ml
β-ESTRADIOL	1.00E-08
LUTEOTROPIC HORMONE	1 µg/ml
DIHYDROTESTOSTERONE	1.00E-10
OTHER ORGANICS	
BOVINE SERUM ALBUMIN	250µg/ml
CHOLERA TOXIN	25 *ng*/ml
CHOLINE CHLORIDE	1.00E-04
D-GLUCOSE	7.00E-03
HYPOXANTHINE	3.00E-05
myo-INOSITOL	1.00E-04
PHOSPHOETHANOLAMINE	5.00E-06
PUTRESCINE	2.00E-06
PYRUVIC ACID (Sodium)	2.00E-03
THYMIDINE	3.00E-06
TRYPSIN INHIBITOR	10 ug/ml
INORGANIC SALTS	
CALCIUM CHLORIDE	2.00E-04
MAGNESIUM CHLORIDE	5.20E-04
MAGNESIUM SULFATE	1.60E-04
POTASSIUM CHLORIDE	3.80E-03
POTASSIUM PHOSPHATE(Mono)	4.30E-04
SODIUM CHLORIDE	1.12E-01
SODIUM PHOSPHATE(Dibasic)	8.90E-04
TRACE ELEMENTS	
CUPRIC SULFATE	1.00E-08
FERROUS SULFATE	3.00E-06
SELENIOUS ACID	3.00E-08
ZINC SULFATE	5.00E-07

Table C. Primers Used in PCR Experiments

PCR for	Primers Used	Reference
pRSVT plasmid which contains the SV40-T antigen gene	combination 1: GCA-TAC-TCT-GTT-ACA-AGC-TTC TCC-AAC-CTA-TGG-AAC-TGA-TG combination 2: GCA-TAC-TCT-GTT-ACA-AGC-TTC GAA-ATG-CCA-TCT-AGT-GAT-GAT-G	Driscoll,K.E., Carter, J.M., Iype, P.T., Kumari, H.L., Crosby, L.L., Aardema, M.J., Isfort, R.J., Cody, D., Chestnut, M.H., Burns, J.L., and LeBoeuf, R.A. In Vitro Cell. Dev. Biol.-Animal 31:516-527, 1995.
p53 gene - exon 8	ACC-TGA-TTT-CCT-TAC-TGC-CTC-TGG-C GTC-CTG-CTT-GCT-TAC-CTC-GCT-TAG-T	Clontech Human p53 Amplimer Panel
H-ras gene - 12th codon	ATG-ACG-GAA-TAT-AAG-CTG-GT CGC-CAG-GCT-CAC-CTC-TAT-A	Clontech Human Ha-ras/12,12 Amplimer Set
H-ras gene - 61st codon	AGG-TGG-TCA-TTG-ATG-GGG-AG AGG-AAG-CCC-TCC-CCG-GTG-CG	Clontech Human Ha-ras/61 Amplimer Set

Table D. Primers Used in RT-PCR Experiments

RT-PCR for	Primers Used	Reference
Cytokeratin 8 (amplified fragment = 277 base pairs)	CTG-GTG-GAG-GAC-TTC-AAG-AAC GAC-CTC-AGC-AAT-GAT-GCT-GTC	Traweek, S.T., Liu, J., and Battifora, H. Am. J. Pathology 142:1111-1118, 1993.
Cytokeratin 18 (amplified fragment = 135 base pairs)	AGC-CAT-TAC-TTC-AAG-ATC-ATC CTC-TGT-CTC-ATA-CTT-GAC-TCT	Traweek, S.T., Liu, J., and Battifora, H. Am. J. Pathology 142:1111-1118, 1993.
BRCA-1 (exon 11) (amplified fragment = 581 base pairs)	GGG-CTG-GAA-GTA-AGG-AAA-CAT-G CAG-GAT-GAA-GGC-CTG-ATG-TAG-G	Gowen, L.C., Johnson, B.L., Latour, A.M., Sulik, K.K., and Koller, B.H. Nature Genetics 12:191-194, 1996.
erbB2 (amplified fragment = 217 base pairs)	CAC-CTG-TGA-GGC-TTC-GAA-GCT-GCA-G GGA-TAT-CCA-GGA-GGT-GCA-GGG-CTA-C	Lonn, U., Lonn, S., Nilsson, B., Stenkvist, B. Cancer 75:2681-2687, 1995.
Epidermal Growth Factor Receptor (amplified fragment = 202 base pairs)	AAT-ATT-CTT-GCT-GGA-TGC-GTT-TCT-GTA TTT-CGA-TAC-CCA-GGA-CCA-AGC-CAC-AGC-AGG	Patel, V.G., Shum-Siu, A., Henifold, B.W., Wieman, T.J.,and Hendler, F.J Am J. Pathology 144:7-14, 1984.

Table E. Background of Viable Breast Tissues Received During the Grant
(Tissues which generated cell strains or cell lines are shown in bold)

Tissue ID	*Race*	*Age*	*Background*	*Tissue From:*	*Pathology*
BRFF029	**Caucasian**	**33**	**Cancer Patient**	**Breast Skin**	**Normal**
BRFF030	Caucasian	69	Cancer Patient	Breast Skin	Normal
BRFF031	Caucasian	57	Cancer Patient	Breast Skin	Normal
BRFF032	Caucasian	34	Cancer Patient	Breast Skin	Normal
BRFF034	Caucasian	52	Cancer Patient	Lymph Node	Metastatic
BRFF035	Caucasian	52	Cancer Patient	Breast	Normal
BRFF036	**Caucasian**	**52**	**Cancer Patient**	**Breast Skin**	**Normal**
BRFF044	Caucasian	74	Cancer Patient	Primary Breast	Carcinoma
BRFF046	Black	51	Cancer Patient	Primary Invasive	Carcinoma
BRFF047	**Caucasian**	**48**	**Cancer Patient**	**Primary Breast**	**Carcinoma**
BRFF049	**Caucasian**	**57**	**Cancer Patient**	**Liver**	**Metastatic**
BRFF050	Caucasian	69	Cancer Patient	Ovary	Metastatic
BRFF051	Black	72	Cancer Patient	Breast	Normal
BRFF052	Black	72	Cancer Patient	Primary Breast	Carcinoma
BRFF053	Black	72	Cancer Patient	Lymph Node	Metastatic
BRFF065	Caucasian	32	Cancer Patient	Lymph Node	Metastatic
BRFF069	**Caucasian**	**55**	**Cancer Patient**	**Lymph Node**	**Metastatic**
BRFF071	**Caucasian**	**44**	**Cancer Patient**	**Lymph Node**	**Metastatic**
BRFF079	**Caucasian**	**57**	**Cancer Patient**	**Breast Skin**	**Normal**
BRFF080	Caucasian	57	Cancer Patient	Primary Breast	Cancer
BRFF081	Caucasian	63	Cancer Patient	Primary Breast	Cancer
BRFF082	**Black**	**27**	**Reduct. Mammoplasty**	**Breast**	**Normal**
BRFF084	Caucasian	71	Cancer Patient	Primary Breast	Cancer
BRFF086	Caucasian	84	Cancer Patient	Primary Breast	Cancer
BRFF087	**Caucasian**	**84**	**Cancer Patient**	**Breast**	**Normal**
BRFF088	**Caucasian**	**40**	**Cancer Patient**	**Breast**	**Normal**
BRFF089	**Caucasian**	**57**	**Cancer Patient**	**Lymph Node**	**Metastatic**
BRFF096	**Caucasian**	**64**	**Cancer Patient**	**Primary Breast**	**Cancer**

Table E. Background of Viable Breast Tissues Received During the Grant

(Tissues which generated cell strains or cell lines are shown in bold)

Tissue ID	*Race*	*Age*	*Background*	*Tissue From:*	*Pathology*
BRFF097	**Caucasian**	**39**	**Reduct. Mammoplasty**	**Breast**	**Normal**
BRFF098	**Caucasian**	**34**	**Reduct. Mammoplasty**	**Breast**	**Normal**
BRFF099	**Caucasian**	**53**	**Cancer Patient**	**Breast**	**Normal**
BRFF100	Caucasian	45	Cancer Patient	Breast	Normal
BRFF103	Black	43	Cancer Patient	Lymph Node	Metastatic
BRFF105	**Caucasian**	**84**	**Cancer Patient**	**Primary Breast**	**Cancer**
BRFF108	Unknown	74	Cancer Patient	Primary Breast	Cancer
BRFF109	**Black**	**59**	**Cancer Patient**	**Primary Breast**	**Cancer**
BRFF110	**Caucasian**	**51**	**Cancer Patient**	**Primary Breast**	**Cancer**
BRFF111	Caucasian	71	Cancer Patient	Primary Breast	Cancer
BRFF115	Caucasian	82	Cancer Patient	Tumor	Cancer
BRFF116	Caucasian	39	Cancer Patient	Primary Breast	Cancer
BRFF117	**Caucasian**	**71**	**Cancer Patient**	**Lymph Node**	**Metastatic**
BRFF118	Caucasian	61	Cancer Patient	Primary Breast	Cancer
BRFF119	Caucasian	61	Cancer Patient	Lymph Node	Metastatic
BRFF120	Caucasian	49	Cancer Patient	Primary Breast	Cancer
BRFF121	Unknown	75	Cancer Patient	Recurrent Tumor	Cancer
BRFF122	Caucasian	55	Cancer Patient	Primary Breast	Cancer
BRFF123	Caucasian	76	Cancer Patient	Brain	Metastatic
BRFF127	Caucasian	49	Cancer Patient	Lymph Node	Metastatic
BRFF128	Caucasian	67	Cancer Patient	Primary Breast	Cancer
BRFF129	**Caucasian**	**67**	**Cancer Patient**	**Lymph Node**	**Metastatic**
BRFF130	**Caucasian**	**61**	**Cancer Patient**	**Lymph Node**	**Metastatic**
BRFF131	Caucasian	60	Cancer Patient	Bone	Metastatic
BRFF132	Caucasian	40	Cancer Patient	Lymph Node	Cancer
BRFF134	Caucasian	39	Cancer Patient	Primary Breast	Cancer
BRFF135	**Caucasian**	**46**	**Cancer Patient**	**Primary Breast**	**Cancer**

Table F. Listing of All Cell Strains and Cell Lines Developed

Cell ID	*Start Date*	*Transfected with Plasmid*	*SC1 Date*	*SC5 Date*	*SC10 Date*	*Cell Cryoed*	*Comments*
BRF029	10/05/1994		10/17/1994	12/06/1994	01/10/1995	T	Cryopreserved
BRF029TA	10/05/1994	pRSVT	10/21/1994			T	Cryopreserved
BRF029TB	10/05/1994	pRSVT	10/21/1994			T	Cryopreserved
BRF029TD	10/05/1994	pRSVT	11/10/1994			F	Discarded contamination
BRF029TF	10/05/1994	pRSVT	12/05/1994			F	Discarded no growth
BRF035TA	10/26/1994	pRSVT	11/17/1994			F	Discarded no growth
BRF035TB	10/26/1994	pRSVT	11/14/1994			F	Discarded no growth
BRF036	10/26/1994		11/17/1994			T	Used for testing
BRF044	12/10/1994		01/09/1995			F	Discarded no growth
BRF046TA	01/24/1995	pRSVT	05/15/1995			F	Discarded no growth
BRF046TB	01/24/1995	pRSVT	03/31/1995			F	Discarded no growth
BRF046TC	01/24/1995	pRSVT	05/09/1995			F	Discarded no growth
BRF047A	01/24/1995		02/13/1995			F	Used for transfection
BRF047B	01/24/1995		02/13/1995			F	Discarded contamination
BRF047C	01/24/1995		02/13/1995			F	Used for transfection
BRF047D	01/24/1995		02/17/1995			F	Used for transfection
BRF047TE	01/24/1995	pRSVT	03/13/1995			F	Discarded no growth
BRF047TG	01/24/1995	pRSVT	02/28/1995			F	Discarded no growth
BRF047TH	01/24/1995	pRSVT	02/28/1995			F	Discarded no growth
BRF047TI	01/24/1995	pRSVT	03/13/1995	05/15/1995		T	Used for testing
BRF049A	02/03/1995		02/17/1995			T	Used for testing
BRF049TD	02/03/1995	pRSVT	02/17/1995			F	Discarded contamination
BRF065A	03/16/1995		04/06/1995			F	Discarded contamination
BRF065B	03/16/1995		04/06/1995			F	Used for transfection
BRF065C	03/16/1995		04/06/1995			F	Discarded contamination
BRF065D	03/16/1995		04/06/1995			F	Discarded contamination
BRF065E	03/16/1995		04/06/1995			F	Discarded contamination

Table F. Listing of All Cell Strains and Cell Lines Developed

Cell ID	*Start Date*	*Transfected with Plasmid*	*SC1 Date*	*SC5 Date*	*SC10 Date*	*Cell Cryoed*	*Comments*
BRF065TF	03/16/1995	pRSVT	04/06/1995			F	Discarded contamination
BRF065TG	03/16/1995	pRSVT	06/13/1995			F	Discarded no growth
BRF069TA	04/04/1995	pRSVT	05/23/1995			F	Discarded redundant
BRF069TB	04/04/1995	pRSVT	05/17/1995			F	Discarded no growth
BRF069TC	04/04/1995	pRSVT	05/23/1995	07/03/1995	08/25/1995	T	Cryopreserved
BRF069TD	04/04/1995	pRSVT	05/17/1995			F	Discarded contamination
BRF071A	04/20/1995		06/06/1995			F	Discarded no growth
BRF071TB	04/20/1995	pRSVT	05/16/1995	06/13/1995	07/17/1995	T	Cryopreserved
BRF071TC	04/20/1995	pRSVT	05/16/1995	06/16/1995	07/17/1995	T	Cryopreserved
BRF071TR6	04/20/1995	T24 DNA	05/16/1995	06/16/1995	04/04/1996	T	Cryopreserved
BRF079TA	07/21/1995	pRSVT	08/14/1995	09/26/1995		T	Cryopreserved
BRF079TB	07/21/1995	pRSVT	08/16/1995			T	Cryopreserved
BRF082A	08/11/1995		08/23/1995			F	Discarded fibroblast
BRF082C	08/11/1995		08/25/1995			F	Used for testing
BRF082TB	08/11/1995	pRSVT	08/28/1995	10/05/1995	11/16/1995	T	Cryopreserved
BRF086A	08/31/1995		09/14/1995			F	Discarded no growth
BRF086TA	08/31/1995	pRSVT	09/12/1995			F	Used for testing
BRF087	08/31/1995		09/11/1995			T	Cryopreserved
BRF088A	08/31/1995		09/11/1995			T	Cryopreserved
BRF088TB	08/31/1995	pRSVT	09/11/1995	11/01/1995		T	Cryopreserved
BRF089	08/31/1995		10/02/1995	11/02/1995		T	Cryopreserved
BRF096A	09/13/1995		09/25/1995			F	Used for testing
BRF096B	09/13/1995		10/19/1995			T	Cryopreserved
BRF096BT	09/13/1995	pRSVT	09/25/1995			F	Discarded no growth
BRF096R	09/13/1995	T24 DNA	10/31/1995			F	Discarded no growth
BRF097C	09/15/1995		10/02/1995			F	Discarded no growth
BRF097TA	09/15/1995	pRSVT	10/12/1995	12/27/1995	02/15/1996	T	Cryopreserved

Table F. Listing of All Cell Strains and Cell Lines Developed

Cell ID	*Start Date*	*Transfected with Plasmid*	*SC1 Date*	*SC5 Date*	*SC10 Date*	*Cell Cryoed*	*Comments*
BRF097TN	03/11/1996		03/29/1996	05/01/1996	06/06/1996	T	Cryopreserved
BRF097TR	09/15/1995	T24 DNA	10/12/1995	12/27/1995	02/15/1996	T	Cryopreserved
BRF098R	09/15/1995	T24 DNA	10/23/1995	12/14/1995		T	Cryopreserved
BRF099	09/15/1995		10/23/1995			F	Used for transfection
BRF099R	09/15/1995	T24 DNA	10/23/1995	12/26/1995		T	Cryopreserved
BRF105B	03/05/1996		07/02/1996			F	Used for testing
BRF105TB	03/05/1996	pRSVT	06/27/1996			T	Cryopreserved
BRF107A	07/08/1996		07/29/1996			F	Used for testing
BRF107B	07/08/1996		07/30/1996			F	Used for testing
BRF107C	07/08/1996		07/30/1996			F	Used for testing
BRF107D	07/08/1996		07/30/1996			F	Used for testing
BRF108B	03/26/1996		04/10/1996			F	Discarded no growth
BRF108TB	03/26/1996	pRSVT	04/16/1996			F	Discarded no growth
BRF109	03/27/1996		04/26/1996	05/28/1996	07/03/1996	T	Cryopreserved
BRF110A	05/09/1996		05/22/1996			F	Used for testing
BRF110B	05/09/1996		05/28/1996	07/03/1996		F	Used for testing
BRF110C	05/09/1996		05/23/1996			F	Used for testing
BRF110TB	05/09/1996	pRSVT	05/28/1996	07/03/1996		T	Cryopreserved
BRF115A	07/10/1996		07/29/1996			F	Used for testing
BRF115B	07/10/1996		07/29/1996			F	Used for testing
BRF115T	07/10/1996	pRSVT	07/29/1996			F	Used for testing
BRF116A	07/16/1996		08/05/1996			F	Used for testing
BRF116B	07/16/1996					F	Used for testing
BRF117	08/01/1996		08/27/1996			T	Cryopreserved
BRF118	08/22/1996		10/03/1996			F	Discarded no growth
BRF119	09/05/1996		10/17/1996			F	Discarded contamination
BRF120T	09/05/1996	pRSVT				F	Discarded no growth

Table F. Listing of All Cell Strains and Cell Lines Developed

Cell ID	*Start Date*	*Transfected with Plasmid*	*SC1 Date*	*SC5 Date*	*SC10 Date*	*Cell Cryoed*	*Comments*
BRF121	09/19/1996					F	Discarded no growth
BRF122	09/26/1996		10/17/1996			F	Used for testing
BRF122	09/26/1996		10/17/1996			F	Discarded contamination
BRF123	10/15/1996					F	Discarded fibroblast
BRF127	10/29/1997					F	Discarded fibroblast
BRF128	11/07/1996	pRSVT	11/26/1996			F	Discarded no growth
BRF129	11/07/1996		12/02/1996			T	Cryopreserved
BRF130	11/26/1996		12/31/1996			T	Cryopreserved
BRF131	01/30/1997					F	Discarded no growth
BRF132T	02/11/1997	pRSVT	03/03/1997			F	Discarded no growth
BRF134T	03/18/1997	pRSVT				F	Used for testing
BRF135	03/20/1997		04/07/1997			F	Used for transfection
BRF135B	03/20/1997		04/17/1997			F	Discarded contamination
BRF135T	03/20/1997	pRSVT	04/10/1997	05/29/1997		T	Cryopreserved

Table G. Details of Tumorigenicity Testing of Breast Cell Lines In Nude Mice

Sex of Mice	*Type of Injection*	*Cell Type Injected*	*Date Injected*	*Duration of Observation in Days*	*No. of Mice with Tumors*
Female	Subcutaneous	MCA-20 P+2[a]	03/14/97	10	4/4
Female	Subcutaneous	BRF-69TC SC10	03/07/97	143	0/5
Female	Subcutaneous	BRF-71TB SC39	02/03/97	143	0/4
Female	Intraperitoneal	BRF-71TB SC39	02/03/97	143	0/1
Female	Subcutaneous	BRF-97TA SC5	01/22/97	155	0/4
Female	Intraperitoneal	BRF-97TA SC5	01/22/97	155	0/1
Female	Subcutaneous	BRF-97TA SC25	03/12/97	138	0/5
Female	Subcutaneous	BRF-97TR SC27	01/23/97	154	0/4
Female	Intraperitoneal	BRF-97TR SC27	01/23/97	154	0/1
Female	Subcutaneous	BRF-97TN SC12	01/21/97	156	0/4
Female	Intraperitoneal	BRF-97TN SC12	01/21/97	156	0/1
Male	Subcutaneous	MCA-20 P+2[a]	03/14/97	10	4/4
Male	Subcutaneous	BRF-69TC SC12	04/03/97	116	0/4
Male	Subcutaneous	BRF-71TB SC39	02/03/97	143	0/5
Male	Subcutaneous	BRF-97TA SC5	01/22/97	155	0/5
Male	Subcutaneous	BRF-97TA SC25	03/12/97	138	0/5
Male	Subcutaneous	BRF-97TR SC27	01/23/97	154	0/4
Male	Intraperitoneal	BRF-97TR SC27	01/23/97	154	0/1
Male	Subcutaneous	BRF-97TN SC12	01/21/97	156	0/4
Male	Intraperitoneal	BRF-97TN SC12	01/21/97	156	0/1

[a] Positive control cells

Table H. Inventory of Breast Cell Strains/Lines at the BRFF Cell Repository

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

Cell Line	Cell Type	SC No.	Date Cryo-preserved	F	R	B	C	R	No. of Units
BRF29	BRST-SKIN	01	10/28/1994	12	03	01	A	1	1
BRF29	BRST-SKIN	01	10/28/1994	12	03	01	A	2	1
BRF29	BRST-SKIN	01	10/28/1994	12	03	01	A	3	1
BRF29	BRST-SKIN	01	10/28/1994	12	03	01	A	4	1
BRF29	BRST-SKIN	01	10/28/1994	12	03	01	A	5	1
BRF29	BRST-SKIN	06	12/27/1994	12	03	01	A	6	1
						****Subtotal****			**6**
BRF29T1	BRST-SKIN	03	11/28/1994	12	03	01	B	1	1
						****Subtotal****			**1**
BRF29T2	BRST-SKIN	03	11/28/1994	12	03	01	B	2	1
						****Subtotal****			**1**
BRF69TC	BREAST	02	06/15/1995	12	03	04	F	4	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	F	5	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	F	6	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	F	7	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	F	8	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	F	9	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	G	1	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	G	2	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	G	3	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	G	4	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	G	5	1
BRF69TC	BREAST	02	06/15/1995	12	03	04	G	6	1
BRF69TC	BREAST	03	10/02/1995	12	03	08	A	8	1
BRF69TC	BREAST	05	01/30/1997	12	03	04	C	3	1
BRF69TC	BREAST	08	02/24/1997	12	03	04	C	4	1
BRF69TC	BREAST	08	02/24/1997	12	03	04	F	3	1
BRF69TC	BREAST	10	03/26/1997	12	03	08	E	8	1
BRF69TC	BREAST	10	03/26/1997	12	03	08	G	9	1
BRF69TC	BREAST	12	04/11/1997	12	03	02	D	2	1
BRF69TC	BREAST	12	04/11/1997	12	03	02	D	3	1
						****Subtotal****			**20**
BRF71TB	BREAST	05	01/15/1997	12	03	03	D	6	1
BRF71TB	BREAST	05	01/15/1997	12	03	03	I	2	1
BRF71TB	BREAST	05	01/15/1997	12	03	03	I	3	1
BRF71TB	BREAST	07	01/30/1997	12	03	05	H	4	1
BRF71TB	BREAST	09	02/10/1997	12	03	05	G	9	1
BRF71TB	BREAST	09	02/10/1997	12	03	05	H	8	1
BRF71TB	BREAST	09	02/10/1997	12	03	05	I	6	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	B	9	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	1	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	2	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	3	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	4	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	5	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	6	1

Table H. Inventory of Breast Cell Strains/Lines at the BRFF Cell Repository

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

Cell Line	Cell Type	SC No.	Date Cryo-preserved	F	R	B	C	R	No. of Units
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	7	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	8	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	C	9	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	1	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	2	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	3	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	4	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	5	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	6	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	7	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	8	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	D	9	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	E	1	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	E	2	1
BRF71TB	BREAST	09	09/17/1997	12	03	06	E	3	1
BRF71TB	BREAST	11	02/19/1997	12	03	09	I	2	1
BRF71TB	BREAST	11	02/19/1997	12	03	09	I	3	1
BRF71TB	BREAST	11	02/19/1997	12	03	09	I	4	1
BRF71TB	BREAST	11	02/19/1997	12	03	09	I	5	1
BRF71TB	BREAST	13	03/05/1997	12	03	08	G	6	1
BRF71TB	BREAST	13	03/05/1997	12	03	08	G	7	1
BRF71TB	BREAST	13	03/05/1997	12	03	08	G	8	1
BRF71TB	BREAST	15	03/21/1997	12	04	01	B	4	1
BRF71TB	BREAST	15	03/21/1997	12	04	01	B	5	1
BRF71TB	BREAST	17	04/08/1997	12	03	02	D	4	1
BRF71TB	BREAST	17	04/08/1997	12	03	02	G	3	1
BRF71TB	BREAST	31	08/02/1996	12	03	05	F	4	1
BRF71TB	BREAST	34	09/06/1996	12	03	09	G	3	1
BRF71TB	BREAST	35	01/10/1997	12	03	02	C	1	1
BRF71TB	BREAST	35	01/10/1997	12	03	02	C	2	1
BRF71TB	BREAST	35	01/10/1997	12	03	02	C	3	1
BRF71TB	BREAST	37	10/03/1996	12	03	01	B	6	1
BRF71TB	BREAST	39	02/18/1997	12	03	09	A	1	1
BRF71TB	BREAST	39	02/18/1997	12	03	09	B	8	1
BRF71TB	BREAST	39	02/18/1997	12	03	09	D	4	1
BRF71TB	BREAST	41	03/04/1997	12	03	08	A	6	1
BRF71TB	BREAST	41	03/04/1997	12	03	08	A	7	1
BRF71TB	BREAST	43	03/19/1997	12	03	08	B	1	1
BRF71TB	BREAST	43	03/19/1997	12	03	08	E	1	1
BRF71TB	BREAST	43	03/19/1997	12	03	08	E	4	1
						****Subtotal****			**54**
BRF71TC	BREAST	04	06/12/1995	12	03	04	C	6	1
BRF71TC	BREAST	04	06/12/1995	12	03	04	C	7	1
BRF71TC	BREAST	04	06/12/1995	12	03	04	C	8	1
BRF71TC	BREAST	04	06/12/1995	12	03	04	C	9	1
BRF71TC	BREAST	04	06/12/1995	12	03	04	D	3	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	F	5	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	F	6	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	F	7	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	F	8	1

Table H. Inventory of Breast Cell Strains/Lines at the BRFF Cell Repository

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

Cell Line	Cell Type	SC No.	Date Cryo-preserved	F	R	B	C	R	No. of Units
BRF71TC	BREAST	10	07/24/1995	12	03	05	F	9	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	1	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	2	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	3	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	4	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	5	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	6	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	7	1
BRF71TC	BREAST	10	07/24/1995	12	03	05	G	8	1
						Subtotal			**18**
BRF71TR	BREAST	08	03/14/1996	12	04	01	C	8	1
BRF71TR	BREAST	08	03/14/1996	12	04	01	C	9	1
						Subtotal			**2**
BRF79TA	BRST-SKIN	04	09/28/1995	12	03	05	H	7	1
						Subtotal			**1**
BRF79TB	BRST-SKIN	02	10/03/1995	12	03	05	H	9	1
						Subtotal			**1**
BRF82TB	BREAST	04	10/05/1995	12	03	03	E	4	1
BRF82TB	BREAST	04	10/05/1995	12	03	03	E	5	1
BRF82TB	BREAST	05	10/12/1995	12	03	03	F	8	1
BRF82TB	BREAST	06	10/19/1995	12	03	08	B	2	1
BRF82TB	BREAST	08	11/07/1995	12	03	05	I	7	1
BRF82TB	BREAST	08	11/07/1995	12	03	05	I	8	1
BRF82TB	BREAST	08	11/07/1995	12	03	05	I	9	1
BRF82TB	BREAST	08	11/09/1995	12	03	08	B	3	1
						Subtotal			**8**
BRF87	BREAST	01	09/25/1995	12	03	05	H	5	1
BRF87	BREAST	01	09/25/1995	12	03	05	H	6	1
						Subtotal			**2**
BRF88A	BREAST	01	09/22/1995	12	03	04	I	8	1
BRF88A	BREAST	02	09/29/1995	12	03	08	A	1	1
BRF88A	BREAST	02	09/29/1995	12	03	08	A	2	1
						Subtotal			**3**
BRF88TBREAST		06	11/15/1995	12	03	08	C	1	1
BRF88TBREAST		07	11/20/1995	12	03	08	C	2	1
						Subtotal			**2**
BRF89	BREAST	02	10/16/1995	12	03	04	F	1	1
BRF89	BREAST	02	10/16/1995	12	03	04	F	2	1
BRF89	BREAST	05	11/10/1995	12	03	08	B	4	1
BRF89	BREAST	05	11/10/1995	12	03	08	B	5	1
BRF89	BREAST	05	11/10/1995	12	03	08	B	6	1
BRF89	BREAST	05	11/10/1995	12	03	08	B	7	1
						Subtotal			**6**
BRF96BBREAST		02	10/09/1995	12	03	03	F	7	1
						Subtotal			**1**
BRF97TA	BREAST	03	12/19/1995	12	03	08	E	5	1
BRF97TA	BREAST	03	12/19/1995	12	03	08	E	6	1
BRF97TA	BREAST	03	07/31/1996	12	03	09	F	6	1

Table H. Inventory of Breast Cell Strains/Lines at the BRFF Cell Repository

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

Cell Line	Cell Type	SC No.	Date Cryo-preserved	F	R	B	C	R	No. of Units
BRF97TA	BREAST	04	12/27/1995	12	03	08	E	9	1
BRF97TA	BREAST	04	12/27/1995	12	03	08	F	1	1
BRF97TA	BREAST	07	02/01/1996	12	03	05	C	8	1
BRF97TA	BREAST	07	02/01/1996	12	03	05	C	9	1
BRF97TA	BREAST	08	03/10/1997	12	03	08	D	3	1
BRF97TA	BREAST	10	02/22/1996	12	03	08	F	9	1
BRF97TA	BREAST	10	02/22/1996	12	03	08	G	1	1
BRF97TA	BREAST	10	02/22/1996	12	03	08	G	2	1
BRF97TA	BREAST	10	02/22/1996	12	03	08	G	3	1
BRF97TA	BREAST	10	02/22/1996	12	03	08	G	4	1
BRF97TA	BREAST	15	05/22/1996	12	03	09	B	5	1
BRF97TA	BREAST	15	05/22/1996	12	03	09	B	6	1
BRF97TA	BREAST	15	05/22/1996	12	03	09	B	7	1
BRF97TA	BREAST	16	01/15/1997	12	03	05	E	3	1
BRF97TA	BREAST	16	01/15/1997	12	03	05	E	4	1
BRF97TA	BREAST	16	01/15/1997	12	03	05	E	5	1
BRF97TA	BREAST	16	01/15/1997	12	03	05	E	6	1
BRF97TA	BREAST	16	01/15/1997	12	03	05	E	7	1
BRF97TA	BREAST	16	01/15/1997	12	03	05	E	8	1
BRF97TA	BREAST	19	02/06/1997	12	03	05	F	1	1
BRF97TA	BREAST	19	02/06/1997	12	03	05	F	2	1
BRF97TA	BREAST	19	02/06/1997	12	03	05	F	3	1
BRF97TA	BREAST	21	02/20/1997	12	04	01	H	2	1
BRF97TA	BREAST	21	02/20/1997	12	04	01	H	3	1
BRF97TA	BREAST	21	02/20/1997	12	04	01	H	4	1
BRF97TA	BREAST	25	03/19/1997	12	04	01	H	5	1
BRF97TA	BREAST	25	03/19/1997	12	04	01	H	6	1
BRF97TA	BREAST	25	03/19/1997	12	04	01	H	7	1
BRF97TA	BREAST	25	03/19/1997	12	04	01	H	8	1
BRF97TA	BREAST	25	03/19/1997	12	04	01	H	9	1
BRF97TA	BREAST	25	03/19/1997	12	04	01	I	1	1
						Subtotal			**34**
BRF97TN	BREAST	00	04/04/1996	12	03	08	H	2	1
BRF97TN	BREAST	00	04/04/1996	12	03	08	H	3	1
BRF97TN	BREAST	00	04/10/1996	12	03	08	I	1	1
BRF97TN	BREAST	01	04/04/1996	12	03	08	H	4	1
BRF97TN	BREAST	01	04/10/1996	12	03	08	H	5	1
BRF97TN	BREAST	01	04/10/1996	12	03	08	H	6	1
BRF97TN	BREAST	01	04/10/1996	12	03	08	H	7	1
BRF97TN	BREAST	01	04/10/1996	12	03	08	H	8	1
BRF97TN	BREAST	01	04/10/1996	12	03	08	H	9	1
BRF97TN	BREAST	06	05/23/1996	12	03	09	C	8	1
BRF97TN	BREAST	06	05/23/1996	12	03	09	C	9	1
BRF97TN	BREAST	06	05/23/1996	12	03	09	D	1	1
BRF97TN	BREAST	06	05/23/1996	12	03	09	D	2	1
BRF97TN	BREAST	09	06/06/1996	12	03	09	D	3	1
BRF97TN	BREAST	09	06/06/1996	12	03	09	D	5	1
BRF97TN	BREAST	09	06/06/1996	12	03	09	D	6	1

Table H. Inventory of Breast Cell Strains/Lines at the BRFF Cell Repository

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

Cell Line	Cell Type	SC No.	Date Cryo-preserved	F	R	B	C	R	No. of Units
BRF97TN	BREAST	09	06/06/1996	12	03	09	D	7	1
BRF97TN	BREAST	09	06/06/1996	12	03	09	D	8	1
BRF97TN	BREAST	09	06/06/1996	12	03	09	D	9	1
BRF97TN	BREAST	09	06/06/1996	12	03	09	E	1	1
BRF97TN	BREAST	10	06/20/1996	12	04	01	G	6	1
BRF97TN	BREAST	10	06/20/1996	12	04	01	G	7	1
BRF97TN	BREAST	10	06/20/1996	12	04	01	G	8	1
BRF97TN	BREAST	15	02/20/1997	12	03	09	I	6	1
BRF97TN	BREAST	15	02/20/1997	12	03	09	I	7	1
BRF97TN	BREAST	15	02/20/1997	12	03	09	I	8	1
BRF97TN	BREAST	15	02/20/1997	12	03	09	I	9	1
						****Subtotal****			**27**
BRF97TR	BREAST	14	03/13/1996	12	04	01	D	1	1
BRF97TR	BREAST	14	03/13/1996	12	04	01	D	2	1
BRF97TR	BREAST	14	03/13/1996	12	04	01	D	3	1
BRF97TR	BREAST	21	05/09/1996	12	03	09	A	2	1
BRF97TR	BREAST	21	05/09/1996	12	03	09	A	3	1
BRF97TR	BREAST	21	05/09/1996	12	03	09	A	4	1
BRF97TR	BREAST	21	05/09/1996	12	03	09	A	5	1
BRF97TR	BREAST	21	05/09/1996	12	03	09	A	6	1
BRF97TR	BREAST	28	02/06/1997	12	03	05	D	4	1
BRF97TR	BREAST	28	02/06/1997	12	03	05	E	9	1
BRF97TR	BREAST	30	02/20/1997	12	04	01	B	7	1
BRF97TR	BREAST	30	02/20/1997	12	04	01	G	9	1
BRF97TR	BREAST	30	02/20/1997	12	04	01	H	1	1
						****Subtotal****			**13**
BRF98RBREAST	02	11/28/1995	12	03	08	C	7	1	
BRF98RBREAST	02	11/28/1995	12	03	08	C	8	1	
BRF98RBREAST	03	12/05/1995	12	03	08	D	1	1	
BRF98RBREAST	03	12/05/1995	12	03	08	D	2	1	
BRF98RBREAST	04	12/14/1995	12	03	08	E	2	1	
BRF98RBREAST	04	12/14/1995	12	03	08	E	3	1	
BRF98RBREAST	05	12/27/1995	12	03	08	F	2	1	
BRF98RBREAST	05	12/27/1995	12	03	08	F	3	1	
BRF98RBREAST	05	12/27/1995	12	03	08	F	4	1	
						****Subtotal****			**9**
BRF99RBREAST	02	12/08/1995	12	03	08	D	5	1	
BRF99RBREAST	03	12/13/1995	12	03	08	D	8	1	
BRF99RBREAST	03	12/13/1995	12	03	08	D	9	1	
						****Subtotal****			**3**
BRF105TB	BREAST	02	07/10/1996	12	03	05	C	2	1
BRF105TB	BREAST	02	07/10/1996	12	03	05	C	3	1
						****Subtotal****			**2**
BRF109 BREAST	01	05/07/1996	12	03	04	C	2	1	
BRF109 BREAST	03	05/13/1996	12	03	09	A	7	1	

Table H. Inventory of Breast Cell Strains/Lines at the BRFF Cell Repository

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

Cell Line	Cell Type	SC No.	Date Cryo-preserved	F	R	B	C	R	No. of Units
BRF109 BREAST	03	05/13/1996	12	03	09	A	8	1	
BRF109 BREAST	03	05/13/1996	12	03	09	B	1	1	
BRF109 BREAST	10	07/09/1996	12	03	09	E	2	1	
						****Subtotal****			**5**
BRF110TB	BREAST	04	07/02/1996	12	03	04	C	1	1
						****Subtotal****			**1**
BRF117 BREAST	01	09/06/1996	12	03	03	D	5	1	
						****Subtotal****			**1**
BRFF125	BREAST	00	09/30/1996	12	04	03	A	1	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	2	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	3	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	4	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	5	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	6	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	7	1
BRFF125	BREAST	00	09/30/1996	12	04	03	A	8	1
BRFF125	BREAST	00	09/30/1996	12	04	03	B	1	1
BRFF125	BREAST	00	09/30/1996	12	04	03	B	2	1
BRFF125	BREAST	00	09/30/1996	12	04	03	B	3	1
						****Subtotal****			**11**
BRF129 BREAST	02	12/31/1996	12	03	02	E	7	1	
						****Subtotal****			**1**
BRF130 BREAST	01	01/30/1997	12	03	02	C	4	1	
						****Subtotal****			**1**
BRF135T	BREAST	01	04/17/1997	12	04	02	D	5	1
BRF135T	BREAST	02	05/12/1997	12	04	02	D	6	1
BRF135T	BREAST	02	05/12/1997	12	04	02	D	7	1
BRF135T	BREAST	03	05/21/1997	12	04	02	D	8	1
BRF135T	BREAST	03	05/21/1997	12	04	02	D	9	1
BRF135T	BREAST	04	05/29/1997	12	04	01	I	2	1
BRF135T	BREAST	04	05/29/1997	12	04	01	I	3	1
BRF135T	BREAST	04	05/29/1997	12	04	01	I	4	1
BRF135T	BREAST	05	06/10/1997	12	04	02	E	1	1
BRF135T	BREAST	05	06/10/1997	12	04	02	E	2	1
						****Subtotal****			**10**

Table I. Inventory of BRFF DNA Bank

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

DNA Isolated From:	SC No.	DNA No.	DNA Isolated On:	μg/ul	μg/vial	F	R	B	R	C	No. Of Vials	
BRF69 Tissue		G-247	01/27/1997	1.300	130.0	PF8	3	1	F	9		1
BRF69 Tissue		G-247	01/27/1997	1.300	130.0	PF8	3	1	G	1		1
BRF69 Tissue		G-247	01/27/1997	1.300	130.0	PF8	3	1	G	2		1
											****Subtotal****	**3**
BRF69TC	6	G-041	08/03/1995	2.700	270.0	PF8	3	1	A	1		1
BRF69TC	6	G-041	08/03/1995	2.700	270.0	PF8	3	1	A	2		1
BRF69TC	6	G-041	08/03/1995	2.700	135.0	PF8	3	1	A	3		1
BRF69TC	6	G-041	08/03/1995	2.700	270.0	PF8	3	1	A	4		1
											****Subtotal****	**4**
BRF71Tissue		G-246	01/21/1997	1.100	55.0	PF8	3	1	F	1		1
BRF71Tissue		G-246	01/21/1997	1.100	55.0	PF8	3	1	F	2		1
BRF71Tissue		G-246	01/21/1997	1.100	55.0	PF8	3	1	F	3		1
BRF71Tissue		G-246	01/21/1997	1.100	55.0	PF8	3	1	F	4		1
											****Subtotal****	**4**
BRF71TB(1)	11	G-042	08/03/1995	0.540	54.0	PF8	3	1	A	5		1
BRF71TB(1)	11	G-042	08/03/1995	0.540	27.0	PF8	3	1	A	6		1
BRF71TB(1)	11	G-042	08/03/1995	0.540	54.0	PF8	3	1	A	7		1
BRF71TB(1)	11	G-042	08/03/1995	0.540	54.0	PF8	3	1	A	8		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	3		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	4		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	5		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	6		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	7		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	8		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	G	9		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	1		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	2		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	3		1

Table I. Inventory of BRFF DNA Bank

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

DNA Isolated From:	SC No.	DNA No.	DNA Isolated On:	μg/ul	μg/vial	F	R	B	R	C	No. Of Vials	
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	4		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	5		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	6		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	7		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	8		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	H	9		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	1		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	2		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	3		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	4		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	5		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	6		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	7		1
BRF71TB(1)	37	G-249	09/26/1997	0.690	25.0	PF8	3	1	I	8		1
											****Subtotal****	**28**
BRF71TC (2)	13	G-059	09/16/1995	0.620	62.0	PF8	3	1	D	4		1
BRF71TC (2)	13	G-059	09/16/1995	0.620	62.0	PF8	3	1	D	5		1
BRF71TC (2)	13	G-059	09/16/1995	0.620	55.8	PF8	3	1	D	6		1
											****Subtotal****	**3**
BRF97 Tissue		G-245	01/14/1997	0.400	40.0	PF8	3	1	F	6		1
BRF97 Tissue		G-245	01/14/1997	0.400	40.0	PF8	3	1	F	7		1
BRF97 Tissue		G-245	01/14/1997	0.400	40.0	PF8	3	1	F	8		1
											****Subtotal****	**3**
BRF97TA	5	G-193	01/11/1996	0.800	80.0	PF8	3	1	E	3		1
BRF97TA	5	G-193	01/11/1996	0.800	80.0	PF8	3	1	E	4		1
BRF97TA	5	G-193	01/11/1996	0.800	80.0	PF8	3	1	E	5		1
BRF97TA	5	G-193	01/11/1996	0.800	80.0	PF8	3	1	E	6		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	A	1		1

Table I. Inventory of BRFF DNA Bank

(F=Freezer; R=Rack; B=Box; R=Row; C=Column)

DNA Isolated From:	SC No.	DNA No.	DNA Isolated On:	μg/ul	μg/vial	F	R	B	R	C	No. Of Vials	
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	B	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	C	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	D	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	E	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	F	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	G	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	H	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	I	1		1
BRF97TA	15	G-250	10/07/1997	1.600	50.0	PF8	3	2	A	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	B	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	C	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	D	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	E	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	F	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	G	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	H	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	I	2		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	A	3		1
BRF97TA	15	G-250	10/07/1997	1.600	320.0	PF8	3	2	B	3		1
											****Subtotal****	**24**
BRF97TN	10	G-243	07/26/1996	1.900	475.0	PF8	3	1	E	9		1
											****Subtotal****	**1**
BRF97TR	17	G-244	11/19/1996	2.100	630.0	PF8	3	1	F	5		1
											****Subtotal****	**1**
BRF99R	5	G-209	02/29/1996	0.810	38.5	PF8	3	1	E	7		1
BRF99R	5	G-209	02/29/1996	0.810	38.5	PF8	3	1	E	8		1
											****Subtotal****	**2**

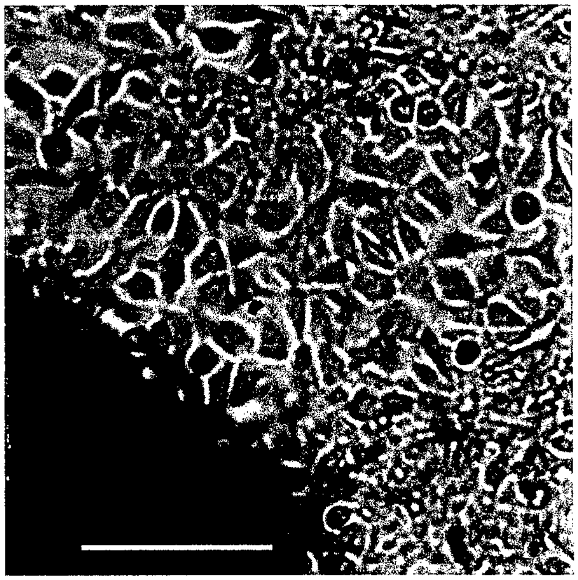


Fig. 1. Light micrograph (without Phase Contrast) of explant cultures of BRF96T. The black area is the tissue attached to an FNC coated dish.

This picture also shows outgrowth of epithelial cells.

The scale bar represents 100 μm.

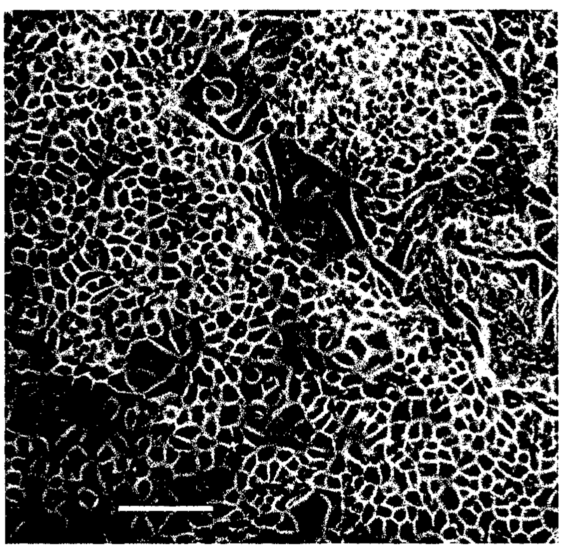


Fig. 2. Phase Contrast Picture of epithelial cells derived from an explant culture of BRF110 tissue.

The scale bar represents 100 μm.

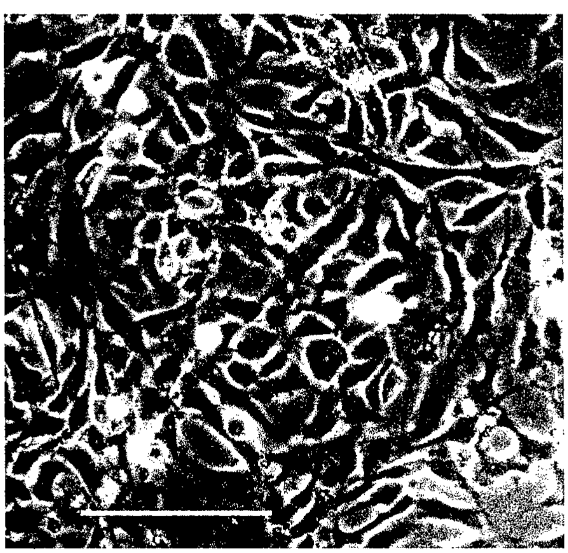


Fig. 3. Phase Contrast Picture of a monolayer culture of BRF69TC at SC4.

The scale bar represents 100 μm.

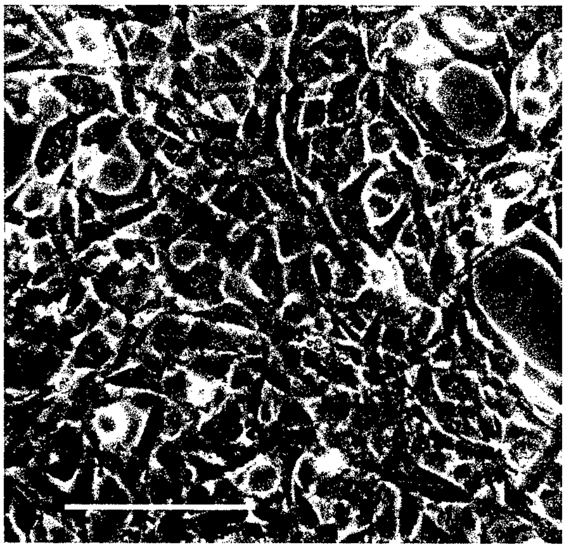


Fig. 4. Phase Contrast Picture of a monolayer culture of BRF71TB at SC35.

The scale bar represents 100 μm.

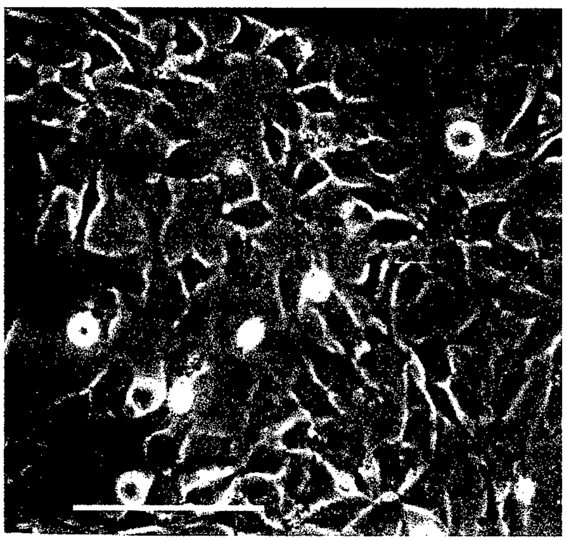


Fig. 5. Phase Contrast Picture of a monolayer culture of BRF71TC at SC6.

The scale bar represents 100 μm.

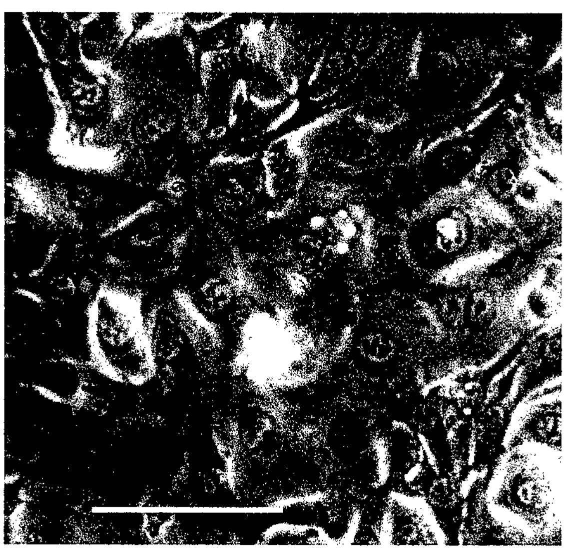


Fig. 6. Phase Contrast Picture of a monolayer culture of BRF82TB at SC10. These cells are relatively large which may be a reflection of their abnormal (triploid) karyotype.

The scale bar represents 100 μm.

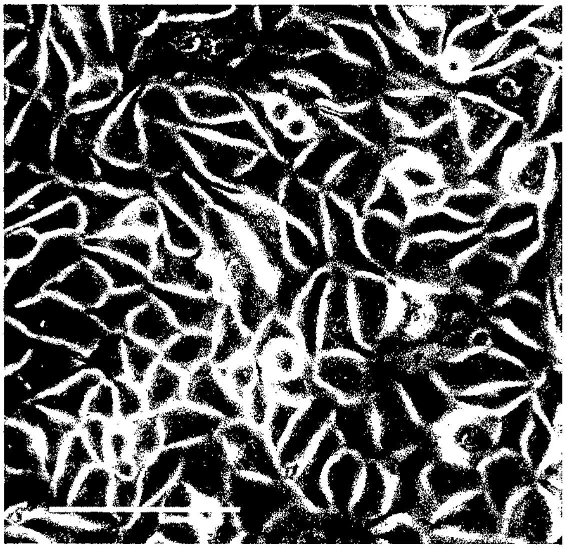


Fig. 7a. Phase Contrast Picture of a monolayer culture of BRF97TA at SC1.

The scale bar represents 100 μm.

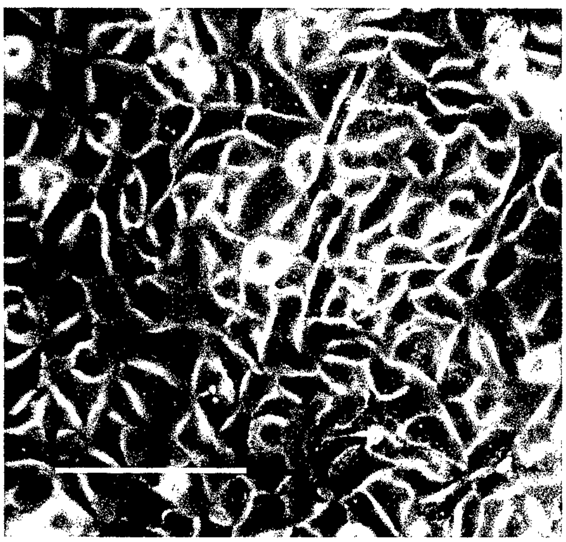


Fig. 7b. Phase Contrast Picture of a monolayer culture of BRF97TA at SC16.

The scale bar represents 100 μm.

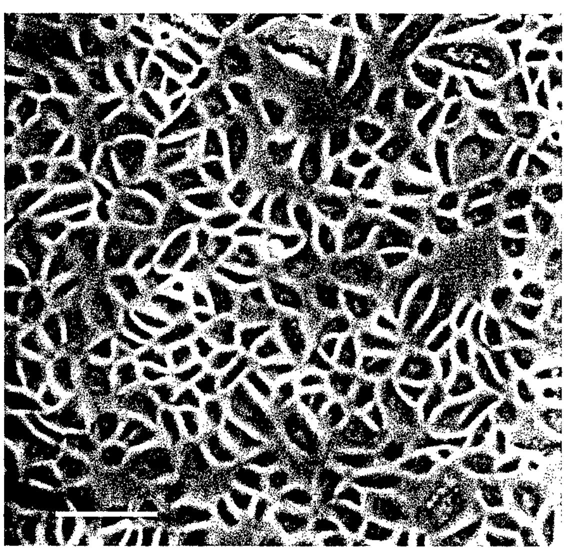


Fig. 8. Phase Contrast Picture of a monolayer culture of BRF99R at SC3.

The scale bar represents 100 μm.

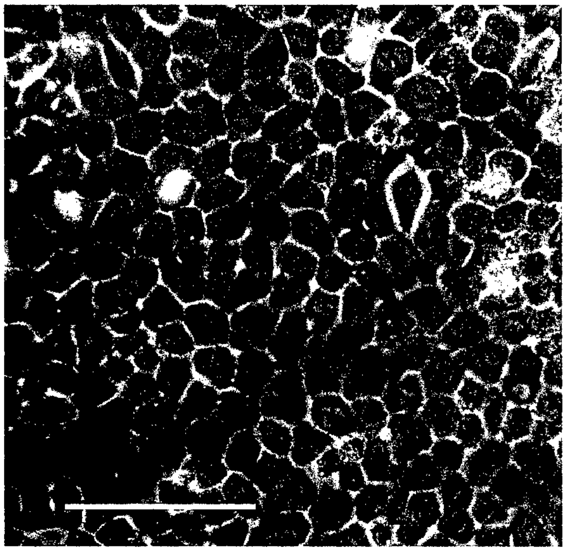


Fig. 9. Phase Contrast Picture of a monolayer culture of BRF97TR at SC23. These cells are smaller than the mother culture, BRF97TA.

The scale bar represents 100 μm.

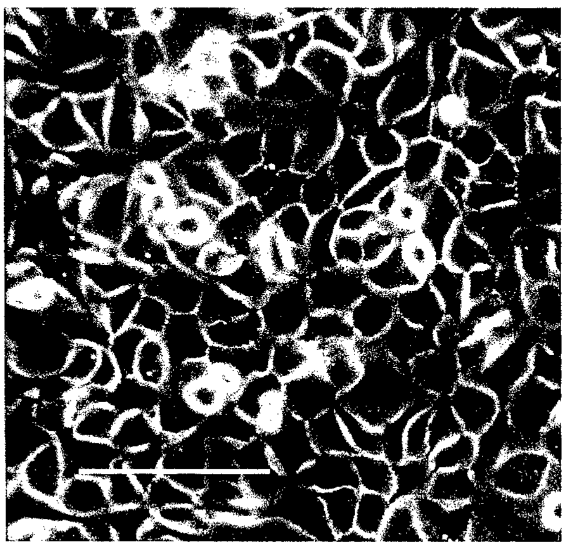


Fig. 10. Phase Contrast Picture of a monolayer culture of BRF97TN at SC24.

The scale bar represents 100 μm.

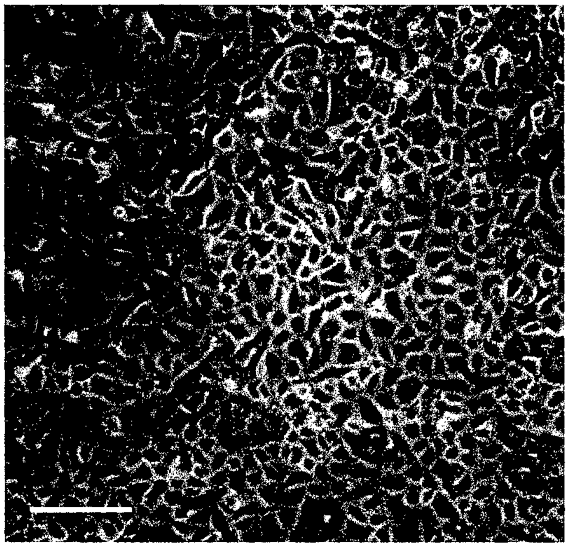


Fig. 11a. Phase Contrast Picture of a mono-layer culture of BRF97TA at SC14 grown in BM0 medium supplemented with 5%FBS. This is a control for BRF97TN below.

The scale bar represents 100 μm.

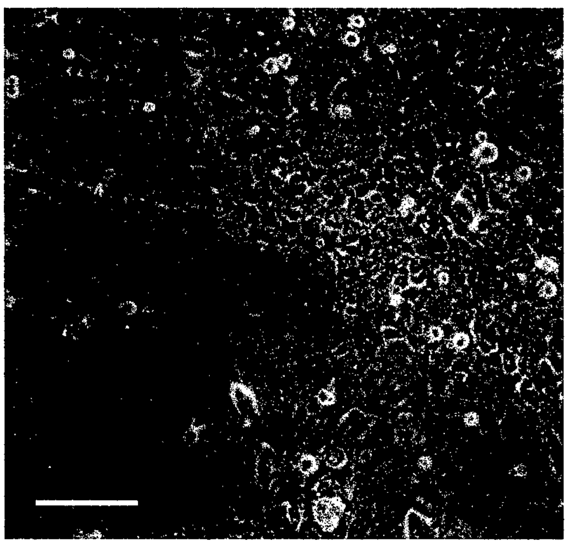


Fig. 11b. Phase Contrast Picture of a mono-layer culture of BRF97TN at SC 0. This culture was derived from BRF97TA (shown above) after treatment with MNNG for 10 days as described in the text.

The scale bar represents 100 μm.

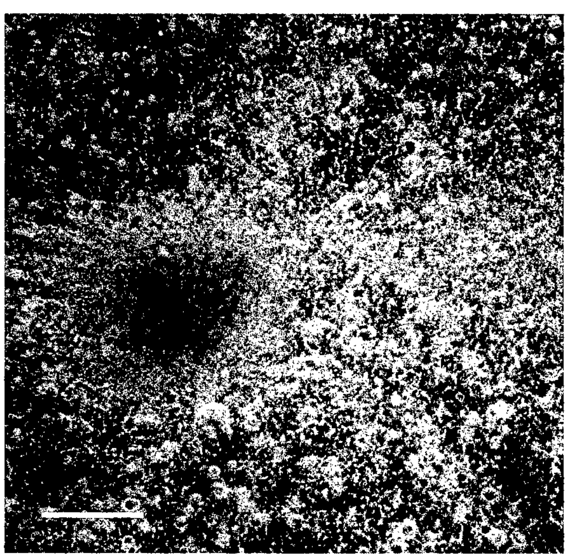
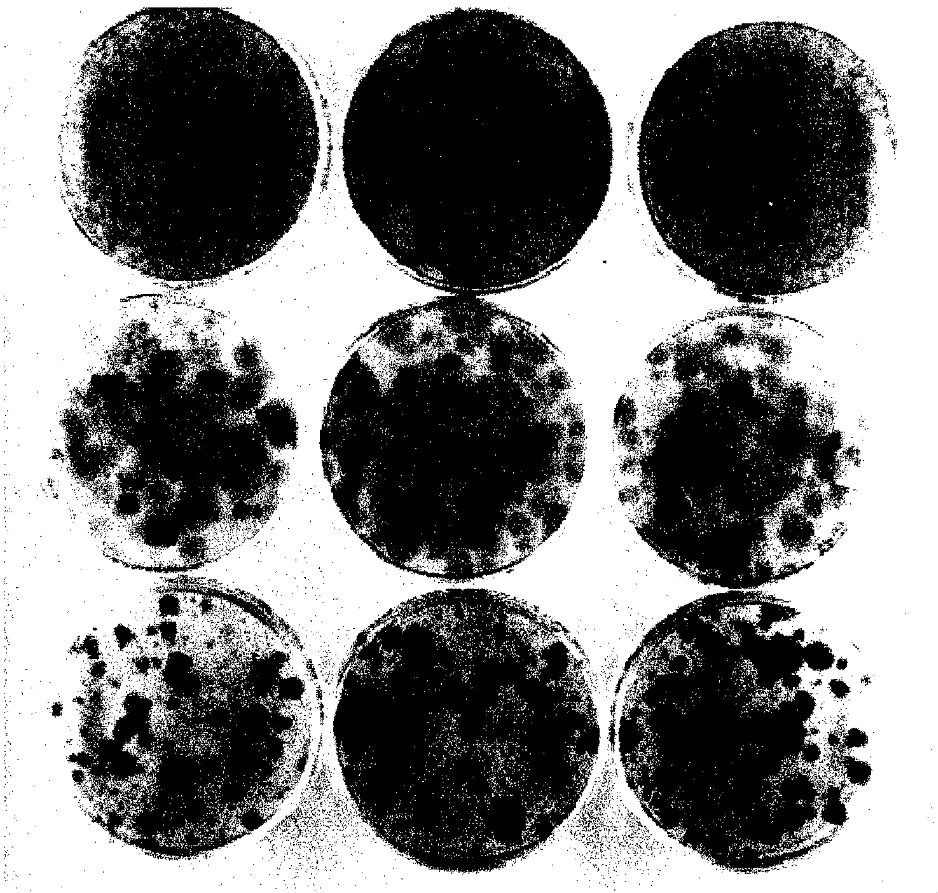


Fig. 11c. Phase Contrast Picture of a mono-layer culture of BRF97TN at SC 0. This culture was derived from BRF97TA (shown above) after treatment with MNNG for 18 days as described in the text. A “piled-up” focus of live cells is seen on the left side of the picture.

The scale bar represents 100 μm.

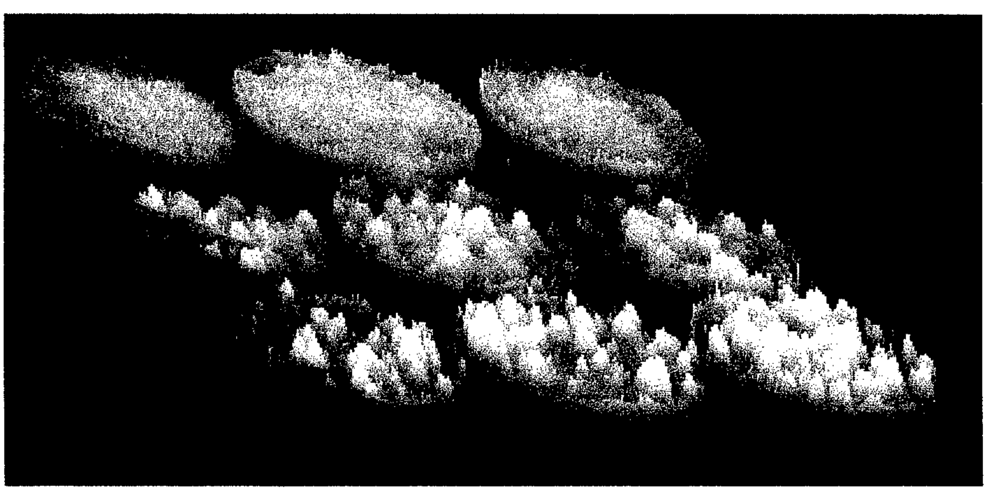
Fig. 12. Morphologically Transformed Foci of BRF97TA Cells.



Untreated cells grown and maintained in BRFF-BM0 + 5% FBS for 2 weeks; Giemsa stained.

MNNG - treated cells grown and maintained in BRFF-BM0 + 5% FBS for 2 weeks; Giemsa stained.

MNNG - treated cells grown and maintained in BRFF-BM0 on FNC-coated dishes for 2 weeks; Giemsa stained.



Above foci as 3-D images photographed with an AMBIS Image Analyzer

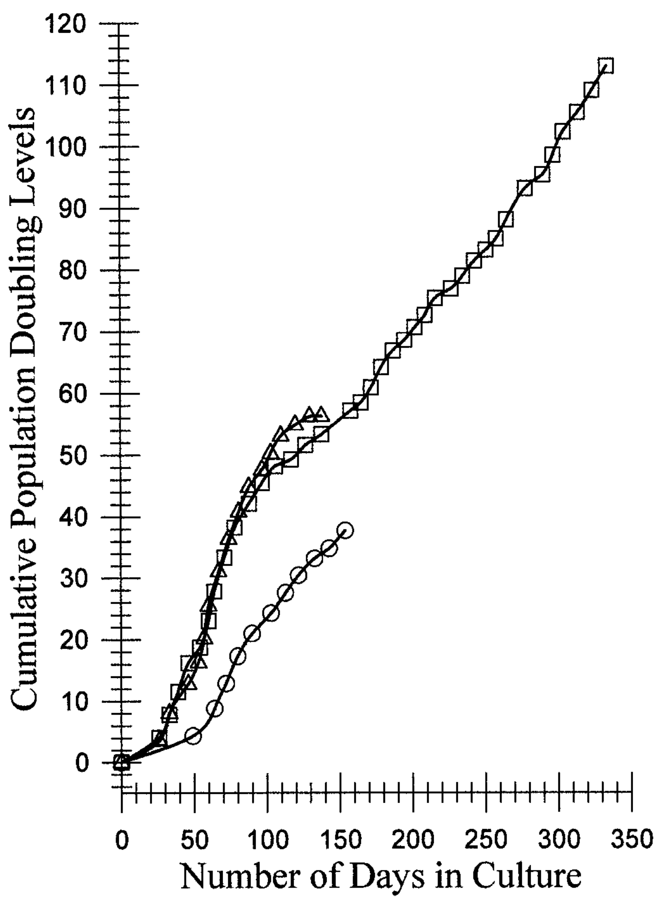


Fig. 13a. Cumulative Population Doubling Levels of human breast cancer cell lines, BRF-69TC, BRF-71TB, and BRF-71TC. The number of cells obtained at each SC was used to calculate CPDL. Population doublings were determined by calculating the fold growth of the cell population (cell number yielded/cell number plated in previous SC). The log of the fold growth was then divided by log2 to give the number of population doublings during that period. Each point on the graph represents a subculture.

BRF-69TC
BRF-71TB
BRF-71TC

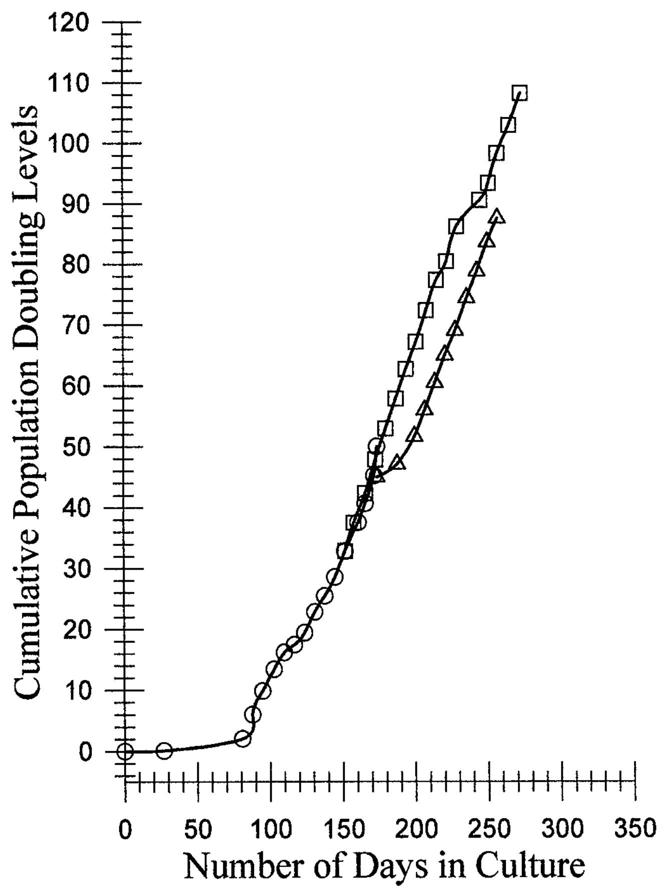
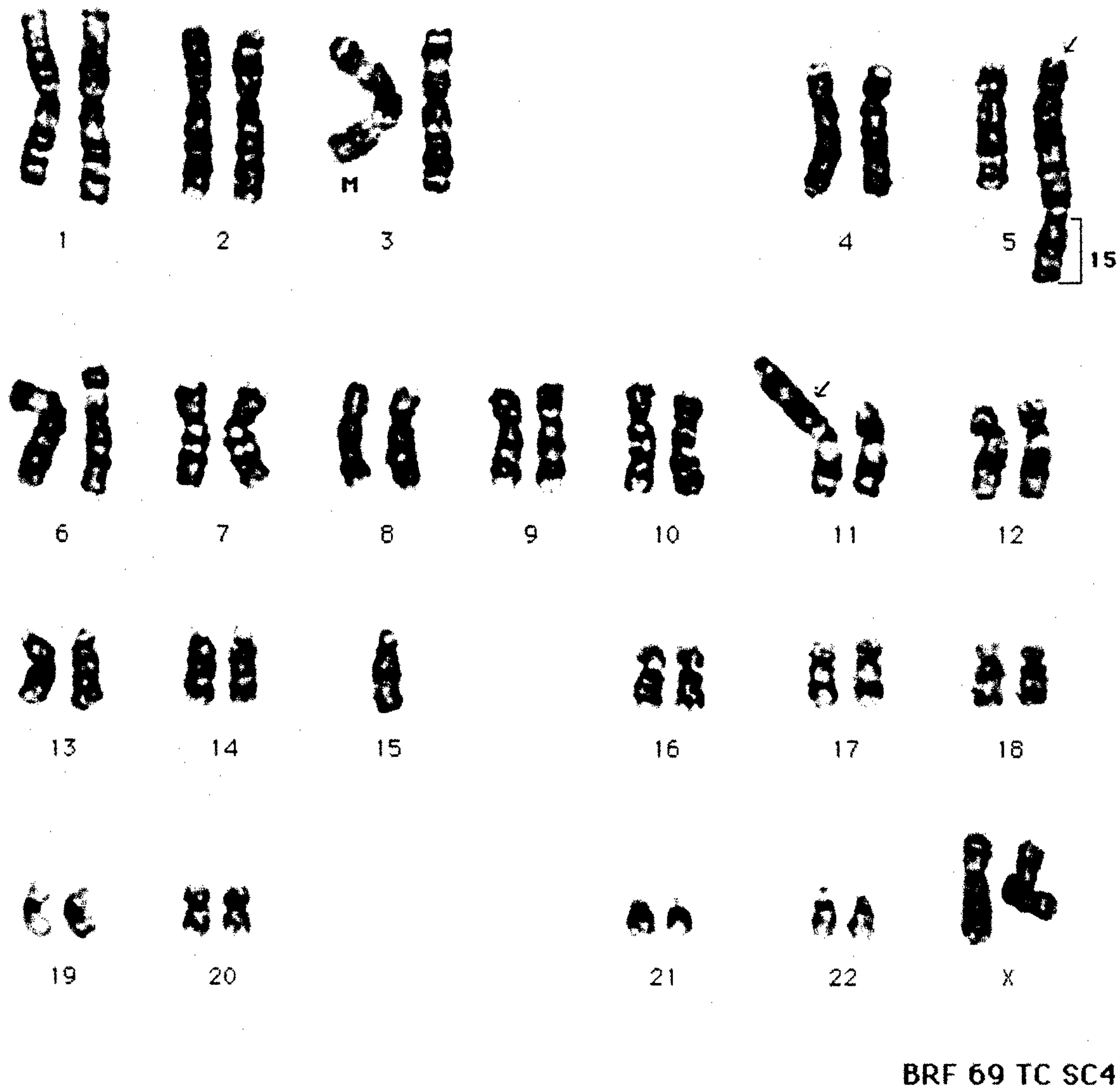


Fig. 13b. Cumulative Population Doubling Levels of a normal human breast cell line, BRF-97TA, and two of its transformed derivatives, BRF-97TR and BRF-97TN. The number of cells obtained at each SC was used to calculate CPDL. Population doublings were determined by calculating the fold growth of the cell population (cell number yielded/cell number plated in previous SC). The log of the fold growth was then divided by log2 to give the number of population doublings during that period. Each point on the graph represents a subculture.

BRF-97TA
BRF-97TR
BRF-97TN

Fig. 14. One of the Giemsa banded karyotypes from BRF69TC at Subculture 4.



BRF 69TC (at SC4) is aneuploid human female (XX), with most chromosome counts in the diploid range. Deletions were observed in one of the #3 chromosomes but, the deleted segment is variable. This deletion was observed in six karyotypes and is marked as M in the Figure.

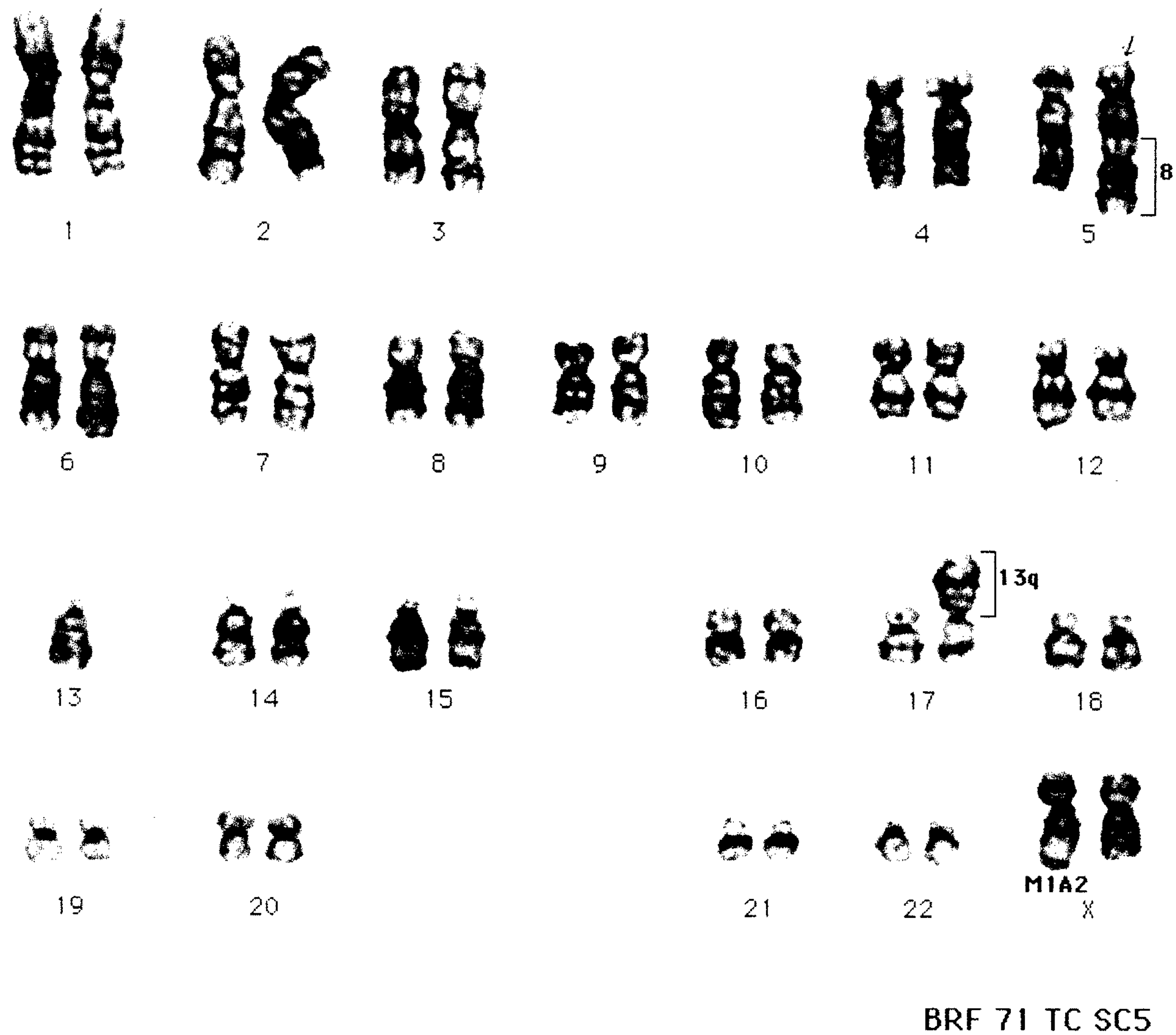
Fig. 15a. One of the Giemsa banded karyotypes from BRF71TB at Subculture 5.

BRF71 TB (at SC5). This cell line is aneuploid human female (XX/XXp-), with most chromosome counts in the diploid range. The aneuploid cells are monosomic for chromosomes 1, 10,12, 16, 18, and/or 21.

Fig. 15b. One of the Giemsa banded karyotypes from BRF71TB at Subculture 38.

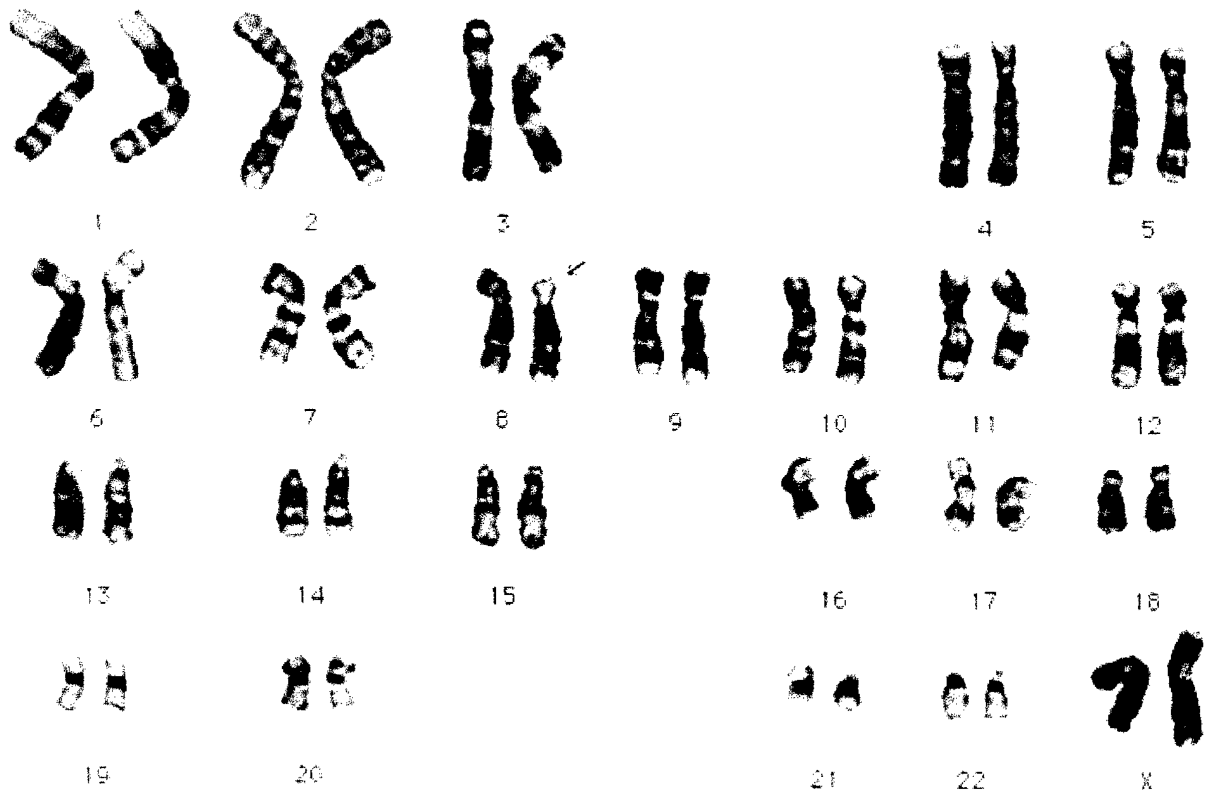
BRF71 TB (at SC38). This cell line is aneuploid human female (XX/XXp/XXp-), with most chromosome counts in the diploid range. Sixteen marker chromosomes were found.

Fig. 16. One of the Giemsa banded karyotypes from BRF71TC at Subculture 5.



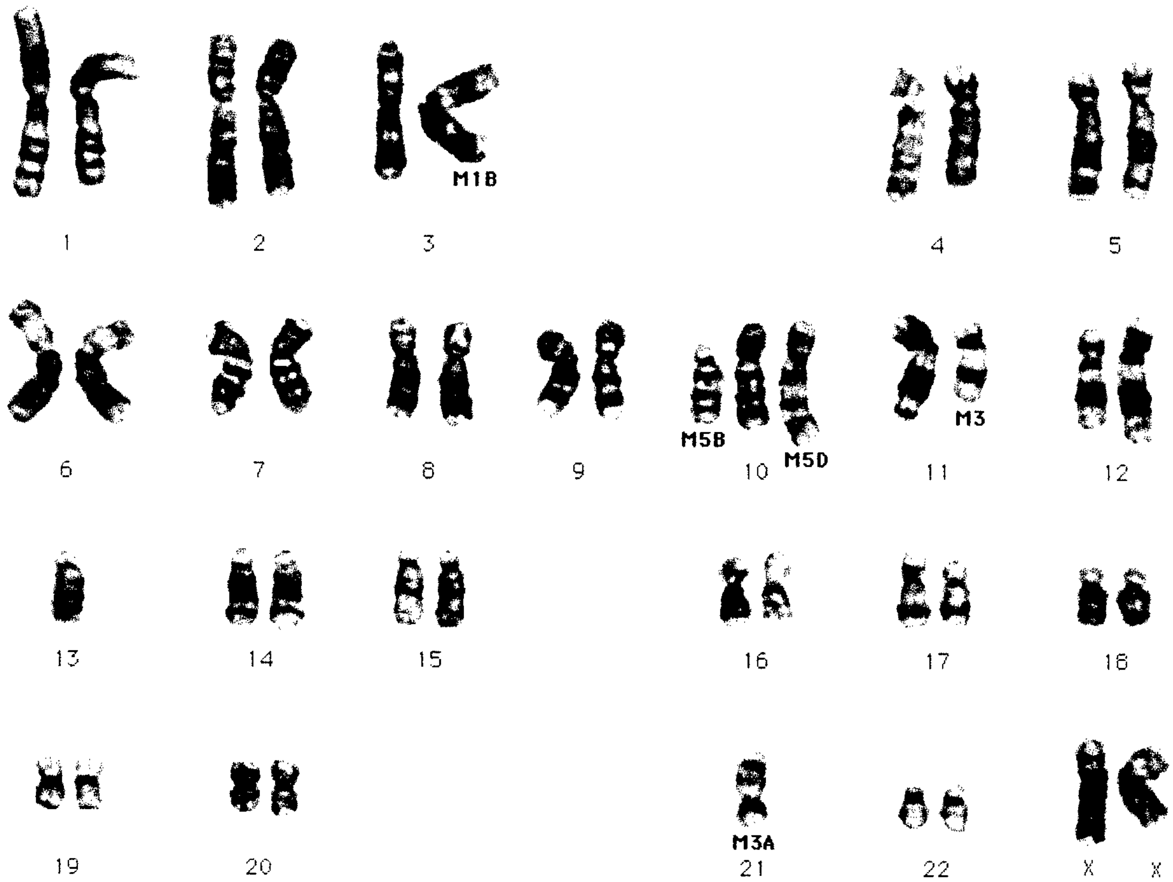
BRF71 TC (at SC5). This cell line is aneuploid human female (XtX), with most chromosome counts in the diploid range. There is one marker chromosome which contain various portions of the missing normal X chromosome.

Fig. 17a. One of the Giemsa banded karyotypes from BRF97TA at Subculture 6.



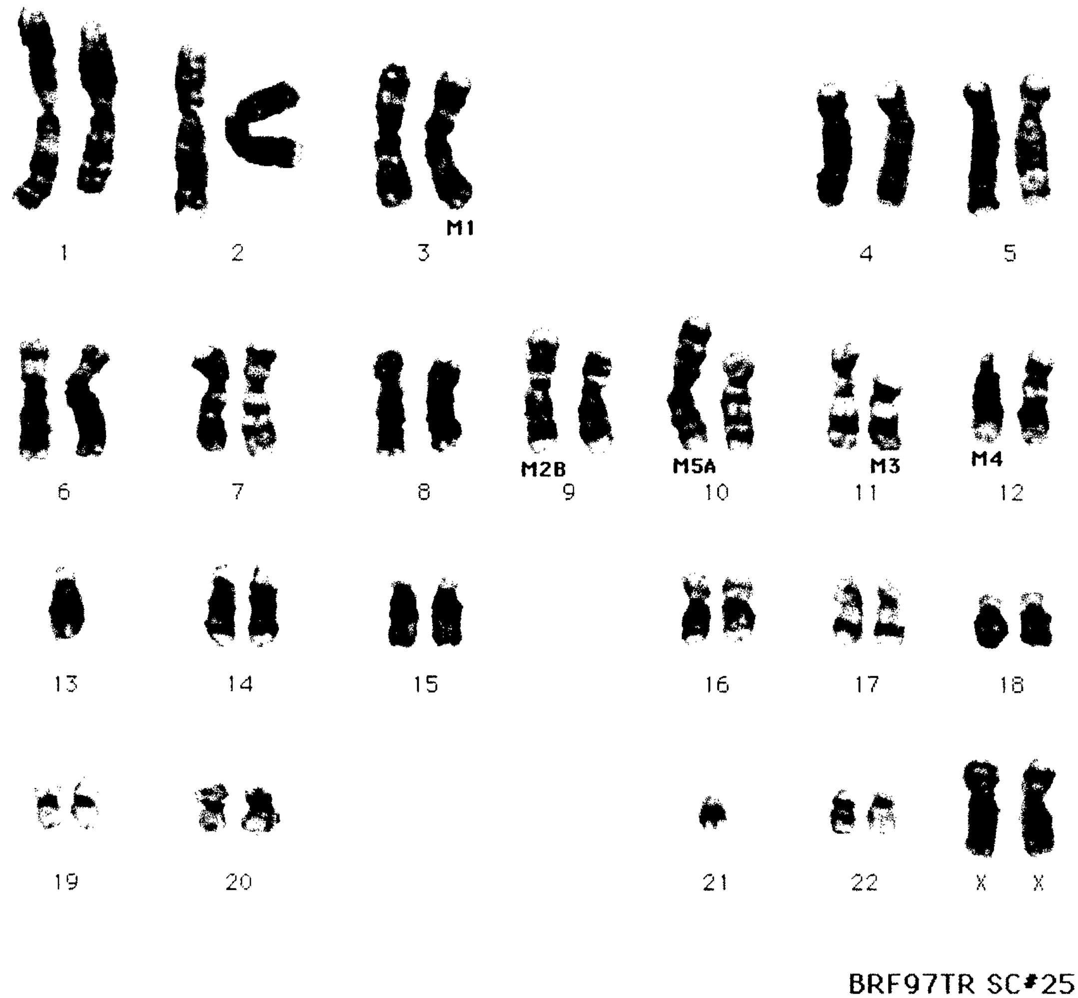
BRF97 TA (at SC6). This cell line is aneuploid human female (XO/XX), with most chromosome counts in the diploid range. There were no markers present.

Fig. 17b. One of the Giemsa banded karyotypes from BRF97TA at Subculture 25.



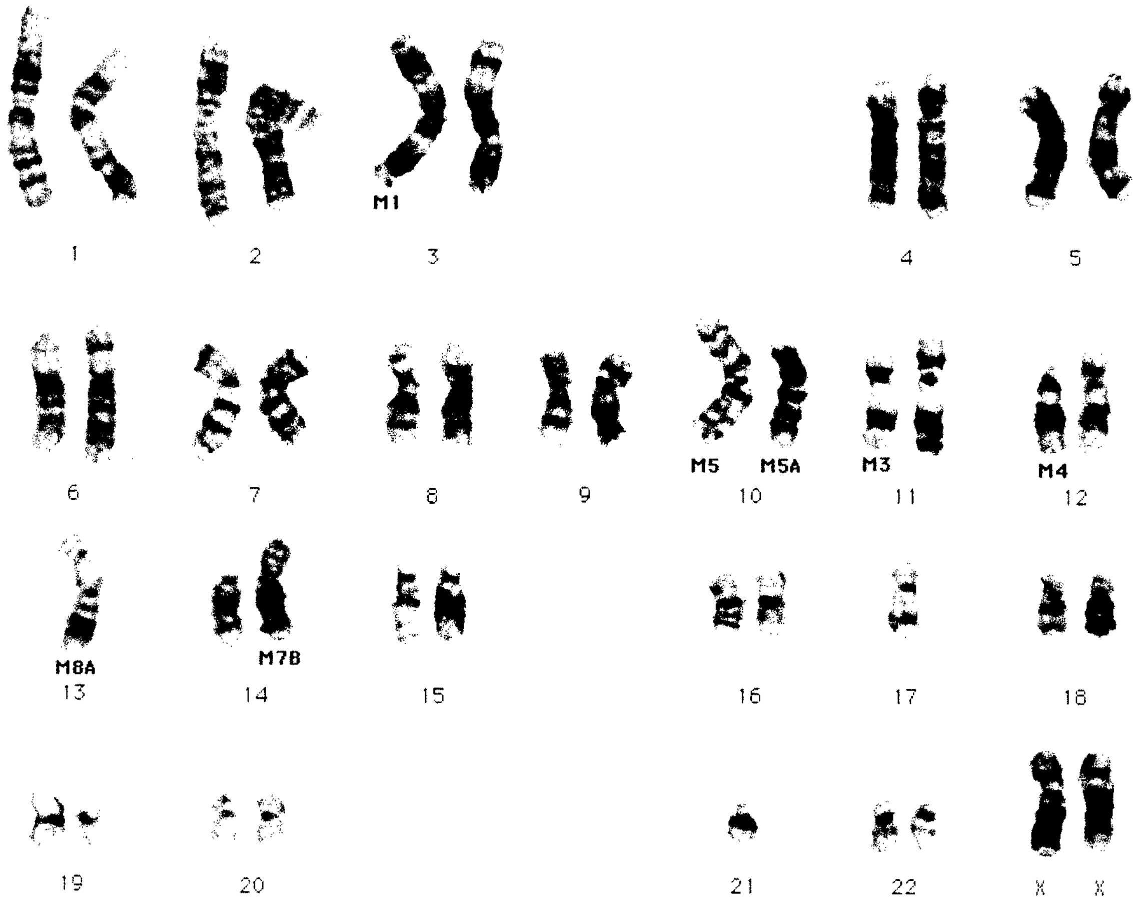
BRF97 TA (at SC25). This cell line is aneuploid human female (XX), with most chromosome counts in the 41-45 range. Twenty-two marker chromosomes were found.

Fig. 18. One of the Giemsa banded karyotypes from BRF97TR at Subculture 25.



BRF97 TR (at SC25). This cell line is aneuploid human female (XX/XO), with most chromosome counts in the hypodiploid range. Seventeen marker chromosomes were found.

Fig. 19. One of the Giemsa banded karyotypes from BRF97TN at Subculture 25.



BRF97 TN (at SC25). This cell line is aneuploid human female (XX/XO), with most chromosome counts in the hypodiploid range. Fifteen marker chromosomes were found.

Fig. 20. Analysis of PCR products generated from human breast cell lines. PCR reactions were performed as described in the text. For each experiment, a negative control (sterile water) and a positive control (0.2 µg pRSVT plasmid for gel A or 0.5 µg human genomic DNA from Clontech for gels B-D) were included. The molecular weight marker is x174 DNA digested with Hae III (Gibco/BRL). In gels A-D, lanes from left to right are: 1.Marker, 2.BRF69TC, 3.BRF71TB, 4.BRF71TC, 5.BRF97TA, 6.Positive Control, 7.Negative Control (A) PCR for the SV40 T-antigen gene. The expected size of the PCR product is 580 bp. (B) PCR for the 12/13th codon of the H-*ras* gene. The expected size of the PCR product is 123 bp. (C) PCR for the 12/13th codon of the K-*ras* gene. The expected size of the PCR product is 111 bp. (D) PCR for exon 8 of the p53 gene. The expected size of the PCR product is 200 bp.

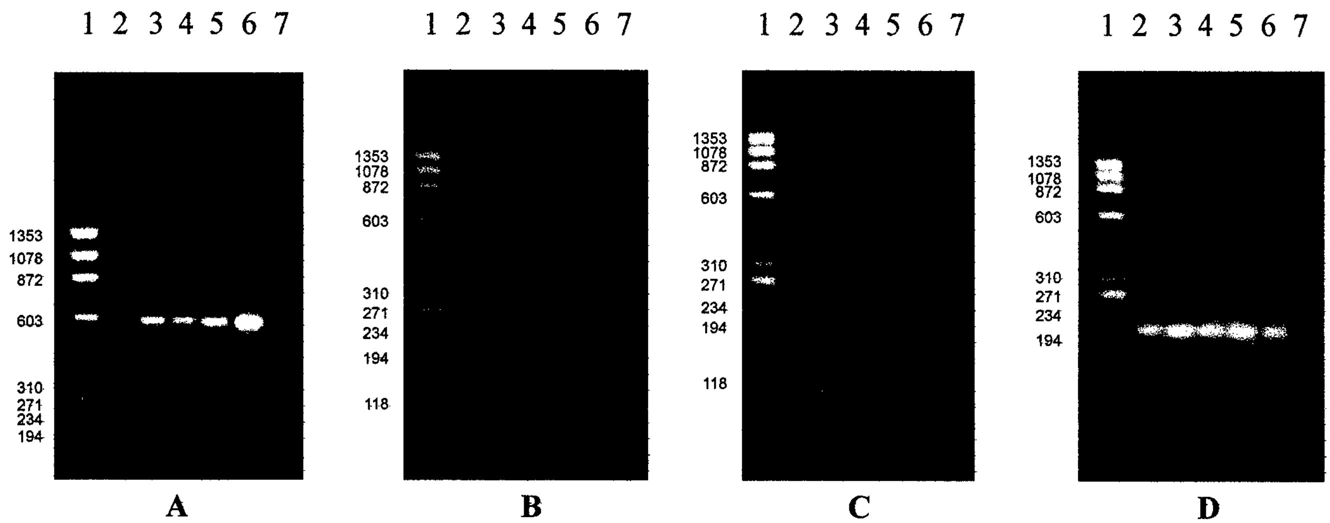
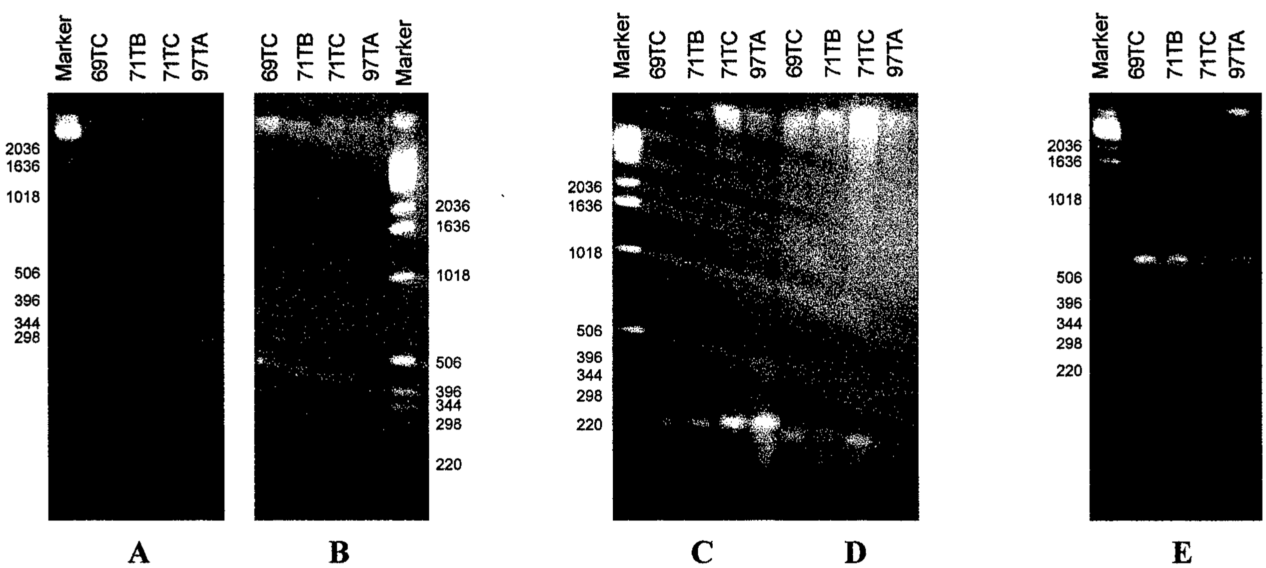


Fig. 21. Analysis of RT-PCR products generated from human breast cell lines. RT-PCR reactions were performed as described in the text. In panels A-E, the molecular weight marker is the 1 kb DNA ladder (Gibco/BRL). The expected size of the RT-PCR products are: cytokeratin 8, 277 bp (A); cytokeratin 18, 135 bp (B); erbB2, 217 bp (C); EGFR, 202 bp (D); and BRCA-1 (exon 11), 581 bp (E).



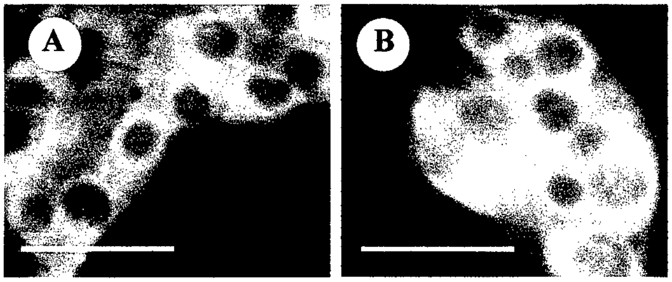


Fig. 22. Indirect immunofluorescent detection of HMFG. (A) BRF71TB at SC39. (B) BRF97TA at SC9.

The scale bars represent 50 μm

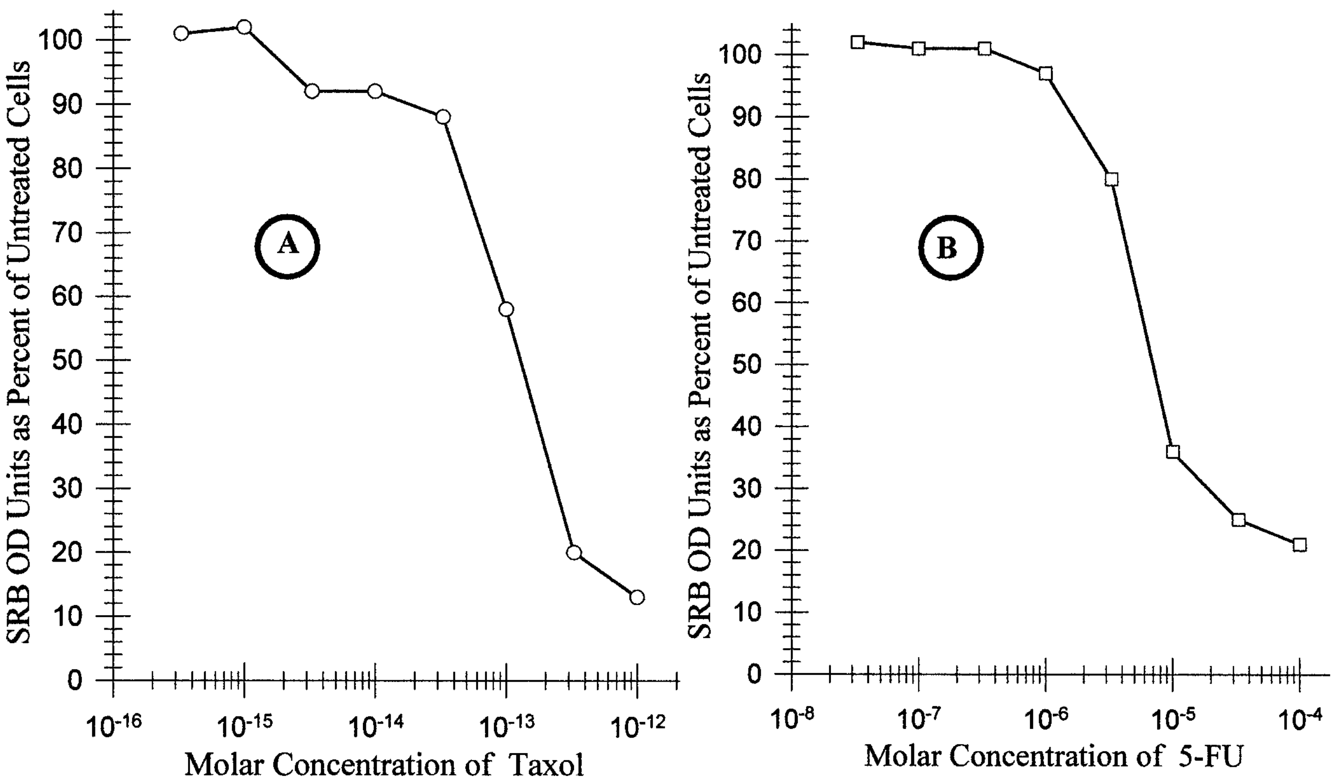


Fig. 23. The effects of two anticancer agents on one of the cancer breast cell lines, BRF71TB. (A) taxol and (B) 5-fluorouracil